X-Ray Powder Diffraction Method Development and Validation for the Identification of Counterfeit Pharmaceuticals

Kelsey DeWitt
dewitt20@live.marshall.edu
(804)814-1762

Internship Agency
U.S. Food and Drug Administration
Forensic Chemistry Center
Trace Examination Section (TES)
6751 Steger Drive
Cincinnati, OH 45237
(513)679-2700

Internship Agency Supervisor – Reviewer 1
JaCinta Batson, M.S.
Supervisory Chemist
Trace Examination Section (TES)
6751 Steger Drive
Cincinnati, OH 45237
(513)679-2700 x 2212
Jacinta.Batson@fda.hhs.gov

Technical Reviewer – Reviewer 2
Mark Witkowski, Ph.D.
Chemist
Trace Examination Section (TES)
6751 Steger Drive
Cincinnati, OH 45237
(513)679-2700 x 2248
Mark.Witkowski@fda.hhs.gov

Technical Reviewer – Reviewer 3
Nicola Ranieri
Biologist
Trace Examination Section
6751 Steger Drive
Cincinnati, OH 45237
(513)679-2700 x 2253
Nicola.Ranieri@fda.hhs.gov

MU Faculty Advisor – Reviewer 4
Lauren Richards-Waugh, Ph.D.
Assistant Professor
Marshall University
Forensic Science Drive, Huntington, WV 25701
(304)691-8940
richards18@marshall.edu

Abstract

Counterfeit pharmaceuticals are illegally manufactured and widely distributed throughout the world, which presents a major threat to public health. Counterfeit pharmaceuticals are unapproved and unregulated products which may contain dangerous or harmful ingredients or an insufficient amount of the active pharmaceutical ingredient (API) patients require to stabilize or improve their health.[5,7] Historically, counterfeit pharmaceuticals have been found to contain the incorrect amount of API, contain a different API, no API, or incorrect excipients within the counterfeit product.[5,7] As the global market place need for pharmaceutical products increases so does the potential for counterfeit products to enter the legal distribution supply chain. Therefore, the FDA's Forensic Chemistry Center is always looking for fast, easy to use and reliable instrumental techniques to screen and identify suspected counterfeit products from authentic products. Thus ensuring that the legal supply chain is secure and maintaining the safety of the public health.[5]

X-Ray Powder Diffraction (XRD) is a technique used in forensic science to analyze various types of trace evidence, such as glass, paint and drugs. XRD has been shown to be a useful technique in the analysis of suspect counterfeit pharmaceutical products.[8,11] Previous work has shown that the x-ray diffraction spectra of authentic pharmaceutical products can be used to compare and differentiate suspect counterfeit products from authentic products. In some cases, this technique can be used to determine the presence or absence of a correct or different API or other excipients within a dosage form.[2,3,7,8] This paper describes the development method for analyzing pharmaceutical solid dosage forms, APIs and excipients using the Bruker AXS D2 Phaser diffractometer (XRD) instrument at the Food and Drug Administration's Forensic Chemistry Center (FCC). The method was validated by measuring the XRD spectra of known

counterfeit pharmaceutical products and comparing them to the XRD spectra of authentic products.

An XRD spectral library was built by analyzing excipients and active pharmaceutical ingredient standards using the Bruker AXS D2 Phaser instrument. Next, authentic pharmaceutical dosage forms were then analyzed and compared to the corresponding XRD spectra of the API and excipient standards to determine if the standards could be observed within the dosage form XRD spectra. The XRD spectral variability between authentic tablets and different lots of authentic product were determined in order to account for any variability observed in the authentic XRD measurements. This was done to determine how potential product changes between tablets and lot numbers of the product could affect the XRD spectra for a given product. Counterfeit dosage forms were then analyzed and compared to the authentic dosage form XRD spectra to determine if the counterfeit products could be differentiated from the authentic products.

Various authentic pharmaceuticals, that are commonly counterfeited, were chosen for this study. The bulk powder excipients and API standards that composed these authentic pharmaceuticals were then selected for analysis. Bulk powder acetylsalicylic acid was selected to be used for sample preparation method experiments because a large abundance of the powder was available for experimentation. All excipients and API standards were prepared and analyzed to be compared with the dosage form spectra. This was conducted to determine if the excipient and API peaks could be distinguishable in the dosage form spectra. Authentic dosage forms were randomly selected for two of the variability studies, Product 7 and 8 were used for the third study because multiple lots were available for these products. The Forensic Chemistry Center

retains known counterfeit products some of which corresponded with the authentic dosage forms used to build the XRD library.

From the results, it was determined that the APIs were not distinguishable in the authentic dosage form XRD spectra, and therefore could not be used to distinguish authenticity as originally assumed. Instead, it was determined that counterfeit products contain different excipients with different crystalline structure than the authentic product. Even in cases where the excipients used in the counterfeit product were the same or similar, this difference in the crystalline structure caused a significant shift in peaks. It was confirmed that a shift greater than 0.2º in the 2θ-diffraction angle is indicative of a counterfeit product.[14] The method developed can be used to distinguish counterfeit pharmaceuticals from authentic pharmaceuticals by examining the overall XRD spectrum for missing peaks, additional peaks and peak shifts.

In addition to developing this method, the parameters needed for a daily performance verification scan to be performed before each use of the instrumentation were being determined. Various measurement setting and statistical evaluation of the new parameters were conducted to ensure that the daily performance scan was within specified limits. More reproducibility studies should be conducted to determine if different analysts could achieve similar XRD spectra results for various dosage form samples. Finally, additional method development studies should be performed to better observe the active ingredient within the dosage forms. A proposed study of extracting and recrystallizing the API from the dosage form could give a more defined spectrum of each API present within the dosage forms.

Introduction

Counterfeit pharmaceuticals are illegally manufactured products that are widely distributed throughout the world, which presents a major public health threat.[5,7,15] These

products often contain dangerous and harmful ingredients which can cause additional illnesses or in severe cases, death. These unregulated products may contain the incorrect active pharmaceutical ingredient (API), may not contain any API, or may contain the correct API at the incorrect dosage strength. Other inactive ingredients, or excipients, could also be added to the product that may cause adverse effects.[1,15] Counterfeits have been found in brand and generic name pharmaceuticals, and have been found in both developing and developed countries. It has been observed that some therapeutic groups of pharmaceuticals may be more likely to be counterfeited than others, such as antibiotics, antihistamines, anti-malarials, hormones, steroids and other therapeutic products.[5]

With these counterfeit products, patients are not receiving the appropriate medication needed to stabilize or improve their health. Many patients will have prolonged illnesses, experience exacerbated symptoms or death as a result from taking a counterfeit medication.[15] If preventative medications, such as anti-malarial pharmaceuticals are counterfeit, the patients taking the counterfeit product are still at risk for developing and spreading the disease. In addition, counterfeit antibiotics with incorrect dosage strength or no API could lead to over prescribed medications, which in turn could lead to antibiotic-resistant bacteria.[15] With this growing public health risk, various techniques and methods continue to be developed to screen and identify these counterfeit pharmaceuticals.

X-Ray Powder Diffraction (XRD) is a fast, reliable and easy to use technique often used in forensic science to analyze various types of trace evidence, such as glass, paint and drugs.[11] Most often x-ray powder diffraction is used for phase identification of various crystalline materials and to gain unit cell dimension information.[6] It has been found that most materials

have distinct XRD spectra and can be identified within a compound or mixture when compared to database of known XRD spectra.[14]

X-ray powder diffractometers consist of several different parts including the cathode ray tube, sample holder, and detector.[6,12,14] The cathode ray tube produces x-rays by heating a metal filament, such as copper, a voltage is then applied to the cathode ray tube causing the x-rays to accelerate toward and bombard the crystalline material at a given angle θ.[6,14] The incident beam is diffracted by multiple layers of atoms of the material creating constructive or destructive interference of the x-ray beams returning to the detector at an angle of 2θ from the incident beam. For the detector to produce an XRD peak, the diffracted beams must have diffraction rays that are in phase, also known as constructive interference. Constructive interference is only produced when the beams fit the criteria of Bragg's Law, $\sin\theta = n\lambda/2d$. A diagram of the diffraction occurring between the different atomic layers of a material can be seen in Figure 1.[12]

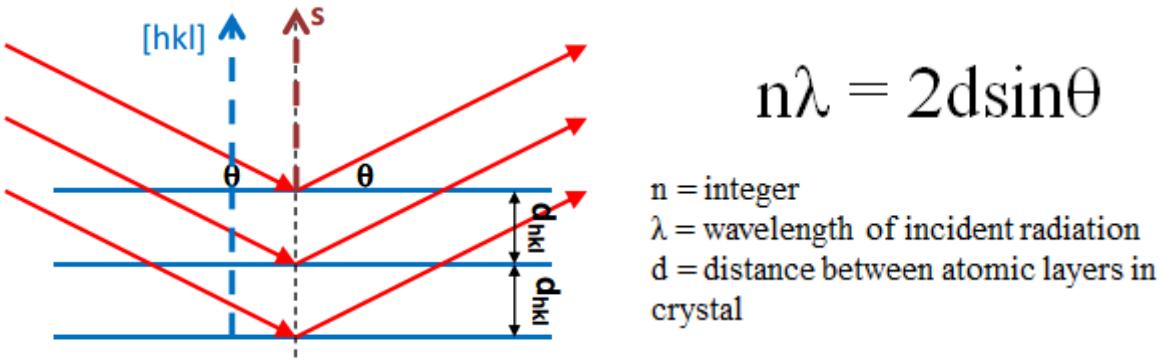

Figure 1. Bragg's Law and a graphical representation of incident x-rays diffracting from atoms within different crystalline layers.[11]

The cathode tube and the detector move to scan the material over a range of 2θ, usually ranging from 5º to 70º for powdered material, but can be extended further to around 140° to ensure that all bands of diffraction are observed. These measurements take into account the random orientations of the different materials that may be in the powdered mixture.[12] Along with the

scan range, the increment size and scan time can also be manipulated. The increment size is the step the detector moves between two points where data is collected and the scan time is the amount of time the detector collects data at a particular point.[10] By decreasing the increment size, more diffraction data can be gathered at various angles, with a larger increment size data may be missed between consecutive angles. A better resolution and an increased signal to noise ratio is a result from a longer scan time, more data can be collected from each particular angle with a large scan time. The disadvantage of these settings is data collection takes a longer period of time. It is recommended to scan a large range at a low resolution with a large increment size to view the overall pattern, and then narrow the range to where the diffraction peaks are visible and increase the resolution of the scan.

The use of x-ray powder diffraction for the identification of counterfeit pharmaceuticals has been performed in various studies.[2,3,7,8] It was found to be a successful technique to identify the presence or absence of APIs within suspect counterfeit pharmaceuticals.[7] Several of these studies have successfully identified the API and other materials without much manipulation or preparation of the dosage form. One study obtained XRD spectra after the removal of the tablet coating[7], while other studies were able to obtain XRD spectra of dosage forms within the blister packaging.[3,9] This non-destructive, low sample preparation technique could be useful when analyzing large numbers of counterfeit pharmaceuticals.

The Food and Drug Administration's Forensic Chemistry Center obtained the Bruker AXS D2 Phaser benchtop x-ray diffractometer and aimed to establish and validate a method for the purpose of identifying counterfeit pharmaceuticals. This paper will describe the method development for the use of this instrument to distinguish a counterfeit from an authentic product.

This project was composed of four sections, first was the development of a daily performance verification procedure to ensure the instrument was functioning within specified limits. Second, sample preparation method experiments were conducted on bulk powder APIs and excipients to achieve optimum results, and then measurements of all APIs and excipients were conducted. Third, sample preparation method experiments were conducted on authentic dosage forms to achieve optimum results, and then measurements of all authentic and counterfeit dosage forms were conducted. Finally, the method was validated based on the guidelines outlined in the USP general chapter<941>[14] to ensure consistent and accurate results for identifying suspect pharmaceuticals as counterfeit.

Materials and Methods

A Bruker AXS D2 Phaser x-ray diffractometer was used to collect the data for this study. The parameters of the diffractometer used throughout the study are in Table 1. Bruker AXS Measurement Suite and Diffrac.EVA V2 were the software programs used on the diffractometer to interpret data and compare spectra, respectively.

Table 1. Bruker D2 Phaser x-ray diffractometer parameters.

Geometry	Bragg-Brentano
Detector	LynxEye™
Scan Type	Coupled Two Theta/Theta
Scan Mode	Continuous PSD Fast
Ceramic X-Ray Tube	KFL Cu-2K
Radiation	Cu-Kα
Generator	Current: 30 kV
	Voltage: 10 mA
Primary Divergence Slit	0.6mm
Axial Soller Slit	2.5°
Receiving Slit	3 mm
Kβ-Filter	Nickel
Cooling Fluid	Innovate
Cooling Fluid Flow Rate	1.6 L/min

Daily Check Parameters

The first study conducted was to determine the best daily check parameters using an aluminum oxide (Al_2O_3) standard reference sample provided by Bruker. This daily check was conducted before every use to ensure that the instrument was running within the specified limits. The manufacturer provided recommended parameters for the daily check, but these were not practical given that each daily check took approximately 55 minutes to complete.[4] For this study, the optimal daily check parameters were run with an increment size of 0.03°, an increment scan time of 0.3 seconds over a scan range of 19-135°, which ran for about 20 minutes. Although the new daily scan was faster, it was experimentally determined that the measurement parameters would meet the specified limits recommended by the manufacturer. These parameters were then run daily for a period of three weeks to generate data for statistical evaluation of the new daily check parameters. The parameters measured were 2θ (peak position) observed, net height and full width half max (FWHM) for four specific peaks. These three parameters were measured at several scan angles, which represent four different peaks associated with the Al_2O_3 XRD spectrum and are shown in Table 2.

Table 2. Daily Check scan angles.[4]

Scan Angles [2θ°]
24.7-26.2
34.1-36.0
88.3-89.7
126.7-129.0

API and Excipient Standards Preparation

After the daily check was performed, various excipients and API standards from a list of authentic products were collected. The list excipients and API standards used throughout this study can be found in Appendix 1. Various sample preparation methods were then performed to determine which method would give the best quality XRD spectra. Bulk powder acetylsalicylic acid was used for the sample preparation method experiments because a large quantity was available for experimental use. Several methods for sample preparation were performed to determine the optimum method for this particular instrument. The individual preparation experiments are listed below:

- Place loose powder on the polymethylmethacrylate (PMMA) sample holder
- use a mortar and pestle to grind the powder and manually press the powder into the PMMA sample holder to create a compact powder
- grind the powder with the mortar and pestle and then compress the powder into a pellet with a Carver pellet press
- grind the powder with the Wig L Bug device and then compress the powder into a pellet with a Carver pellet press

A full scan from 12° to 140° with an increment size of 0.03°, and a scan increment time of 0.1 seconds was then conducted. The angle range was narrowed to fit the particular API or excipient being analyzed. For example if a material did not exhibit any peaks beyond 70°, the second measurement would be scanned between 12° to 70°. The measurement parameters of the second measurement had an increment size of 0.02° and a scan increment time of 0.5 seconds, this improved the signal to noise ratio for the overall XRD spectrum. Each API and excipient were prepared and analyzed using the method that gave the best overall x-ray powder diffraction spectrum.

Authentic and Counterfeit Dosage Forms Sample Preparations

Various authentic dosage forms were chosen and collected for analysis. Multiple sample preparation methods were conducted for authentic dosage forms to determine which method would produce the optimum results. The experiments performed were:

- To analyze the whole authentic dosage form, no manipulations
- To removing the authentic dosage form coating
- To manually grind the dosage form, grind the powder with the Wig-L-Bug, then press the powder into a pellet with the Carver press

Once the optimum sample preparation method was established, various measurements were conducted to determine the variability and reproducibility of spectra collected from authentic dosage forms. The variability within a single tablet and between tablets of the same lot was observed by forming multiple pellets from one tablet and then forming pellets from different tablets. Pellets were also made to determine how the product differs between lots. The XRD spectra produced from the authentic dosage forms were then compared with the excipients and API standards. The XRD spectra were overlaid to visually compare the various spectra of authentic products and standards to try and determine if individual API and excipient peaks could be observed in the finished dosage form. Once all authentic dosage forms were analyzed, known counterfeit dosage forms were then prepared with the same optimal sample preparation method as the authentic dosage forms. Counterfeit spectra were then compared to the authentic spectra of the corresponding product to determine if the XRD spectra could be used to distinguish a counterfeit.

Results

Establishment of Daily Performance Check

An example of Al_2O_3 daily check spectrum using the data collection parameters, which were set to have a scan increment size of 0.03°, a scan time at each increment of 0.3 seconds, and a scan angle range of 19° to 135°, is shown in Figure 2.

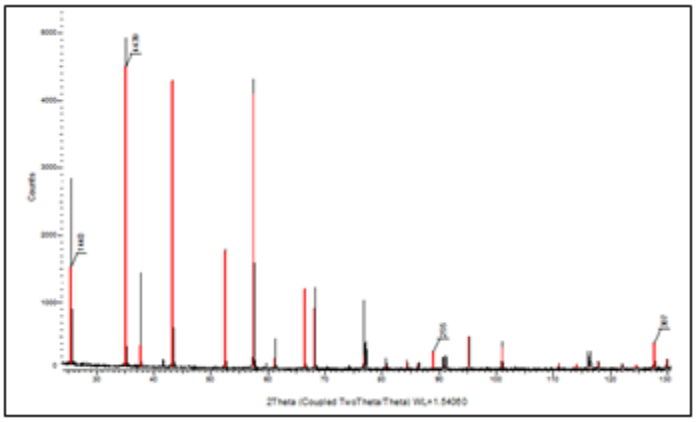

Figure 2. Daily Check scan with the targeted peaks assigned with peak search values assigned by the diffractometer software.

The statistical evaluations are shown in Table 3. Average, standard deviation (STDEV) and percent relative standard deviation (%RSD) were calculated for the observed (measured) 2θ, the net height and FWHM at each of the targeted peaks listed in Table 2. The averages of the 2θ, net height and FWHM were then compared to the expected values given by the manufacturer, shown in Table 4.

Table 3. Statistical evaluation of the new Daily Check parameters.

Daily Check Parameters								
Increment Time:					0.3 sec			
Increment Size:					0.03°			
2θ Observed			**Net Height**			**FWHM**		
Average	**STDEV**	**%RSD**	**Average**	**STDEV**	**%RSD**	**Average**	**STDEV**	**%RSD**
25.583	0.003	0.013	55.153	2.097	3.802	0.062	0.003	4.467
35.148	0.002	0.005	96.412	2.530	2.624	0.064	0.002	3.341
88.965	0.003	0.003	5.525	0.281	5.090	0.107	0.006	5.451
127.662	0.006	0.005	7.269	0.214	2.947	0.176	0.010	5.619

Table 4. Comparison of calculated average at 2θ, net height, and FWHM with the expected values.

2θ (±0.02°)		**Net Height (± 10%)**		**FWHM (FWHMobs ≤ FWHMmax)**	
Calculated	Expected	Calculated	Expected	Calculated	Expected
25.583	25.559	55.2	51.9	0.062	0.086
35.148	35.129	96.4	101.4	0.064	0.079
88.965	88.967	5.5	10.3	0.107	0.109
127.662	127.659	7.3	24.1	0.176	0.180

Sample preparations were then conducted on the bulk powder acetylsalicylic acid to determine the preparation that would result in the optimum XRD spectra. The preparation method would then be used for the preparation of excipients and APIs. It was determined that placing loose powder on the sample holder would not be practical, eliminating this preparation. The second preparation attempted was the use of the mortar and pestle to grind the powder and then press the powder into the sample holder, creating a compact powder. This was conducted using different amounts of standard to determine the amount needed for optimum results. Figure 3 shows data collected from this preparation method.

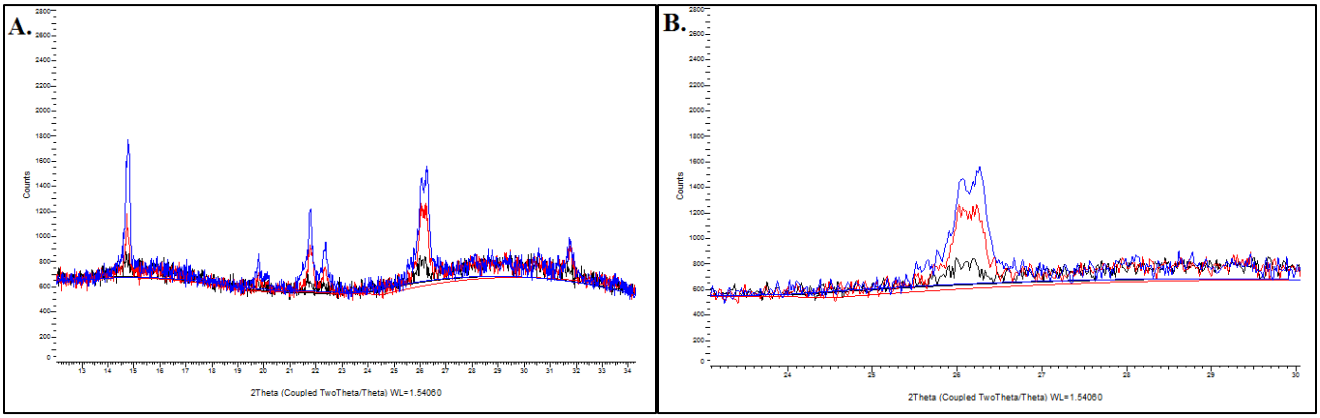

Figure 3. X-ray powder diffraction spectra of various amounts of acetylsalicylic acid overlaid using the mortar and pestle method to create a compact powder in the sample holder. 10mg in black, 30mg in red and 40mg in blue. A contains an excerpt of the full spectrum. B. contains an excerpt of Graph A of the peak occurring between 24° and 30°.

The third preparation was to grind the excipient or API standard powder with the mortar and pestle, and then the Carver press was used to create a pellet. Different amounts of the powder were used to determine the best amount of powder required for the optimum results. Figure 4 shows data collected from the third preparation method.

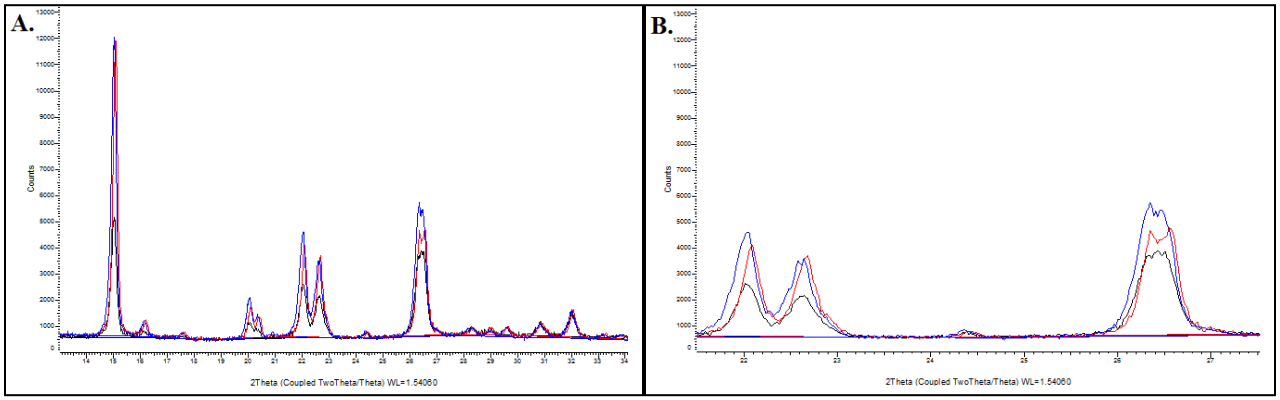

Figure 4. X-ray powder diffraction spectrum of various amounts of acetylsalicylic acid overlaid using the mortar and pestle and Carver press. 20mg in black, 40mg in red, and 60mg in blue. Graph A contains an excerpt of the full XRD spectrum and graph B contains an excerpt of Graph A of the peak occurring between 21.5° and 27.5°.

The results from the second method were then compared to the method using only the mortar and pestle to create a compact powder. The x-ray powder diffraction spectra are overlaid in Figure 5 to compare the two methods. The peak shifting between spectra were determined to be the result of the different compacting methods.

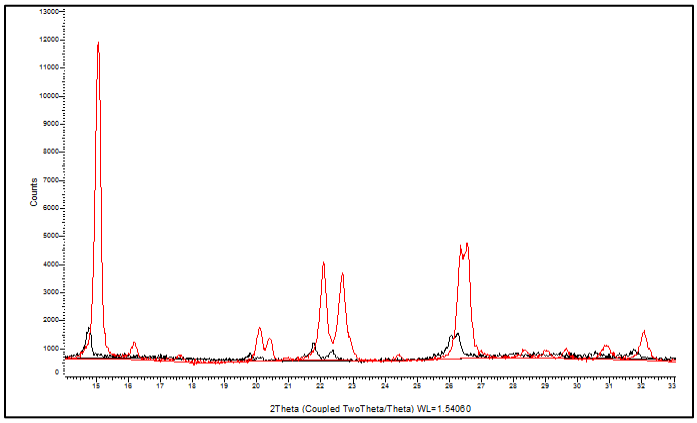

Figure 5. Mortar and pestle method to press the powder into the sample holder (black spectrum) compared to the method with mortar and pestle and Carver pellet, (red spectrum). Each spectrum was produced by 40 mg of acetylsalicylic acid.

The fourth preparation was to grind the standard product with the Wig-L-Bug device then to use the Carver press to create a pellet. The XRD spectra of the pellets created with different grinding methods, mortar and pestle or Wig-L-Bug device, were then compared. The spectra of both can be seen in Figure 6. Unlike Figure 5, peak shifting between spectra is minimal because the compaction method was the same, the Carver press was used at the same pressure to create a pellet for both spectra. From this Figure it was determined that this preparation method would give the optimal results and be used throughout the remainder of the study.

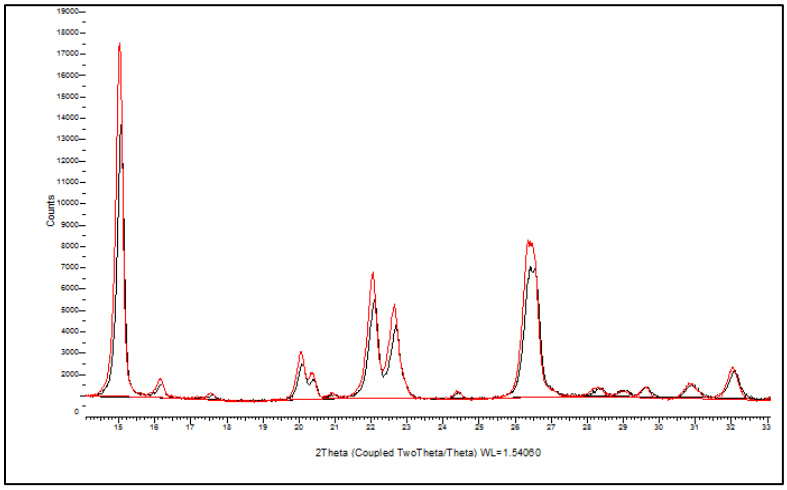

Figure 6. X-ray powder diffraction spectra of 60 mg acetylsalicylic acid pellets with different grinding methods. Mortar and pestle in red and Wig-L-Bug in black.

Data Collection

Once the sample preparation method was determined, x-ray powder diffraction spectra were collected for all bulk powder excipients and API standards that can be seen in Appendix 1. Excipients such as titanium oxide rutile and titanium oxide anastase were compared to literature spectra to determine if the x-ray powder diffraction spectra produced from the Bruker D2 Phaser were accurate.[13] The literature spectra and the two excipient spectra are shown in Figure 7.[13]

The two forms of titanium dioxide, rutile and anatase, were chosen because it was known that each possessed a different crystalline structure. The two were analyzed to observe how the different crystalline structures would vary the XRD spectra.

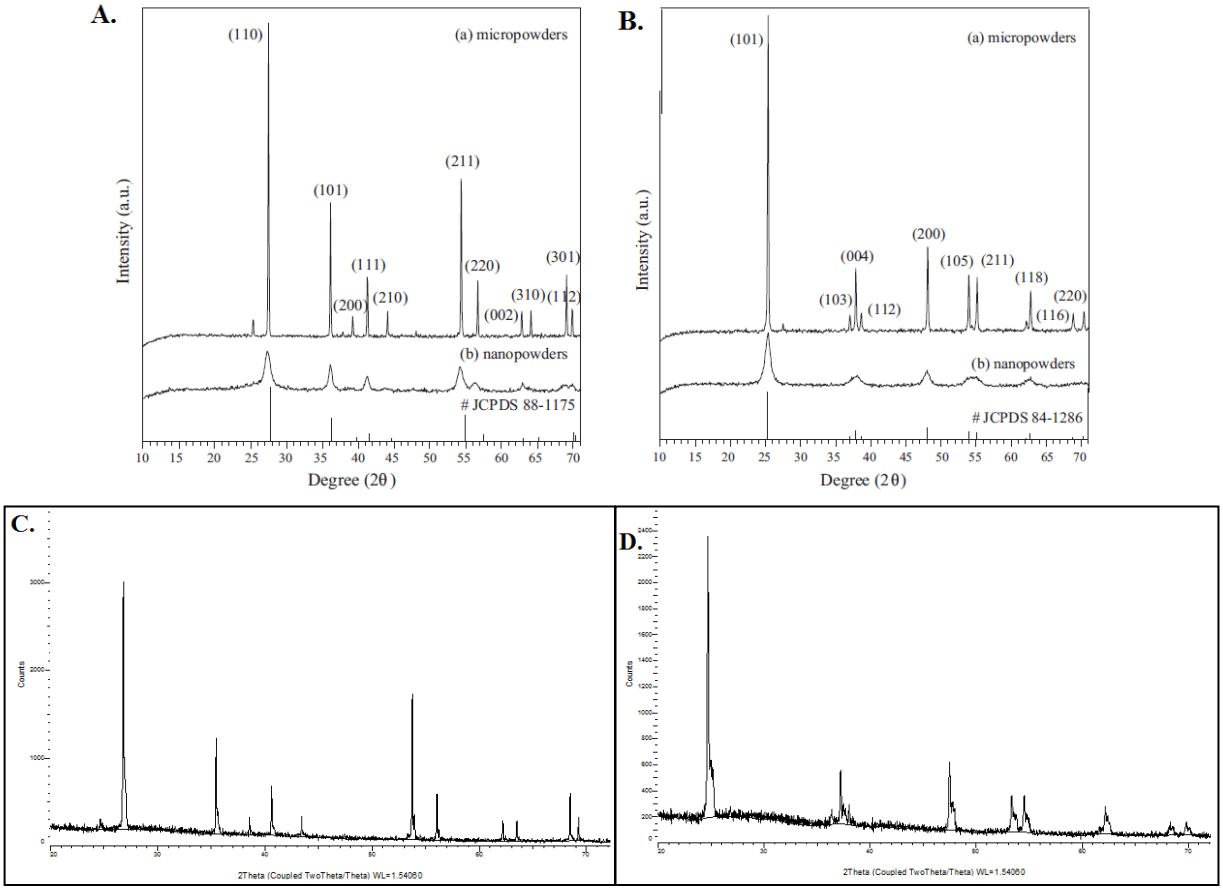

Figure 7. A. Literature XRD spectrum of rutile TiO_2. B. Literature XRD spectrum of anatase TiO_2. C. XRD spectrum of rutile TiO_2 collected with Bruker D2 Phaser during this study. D. XRD spectrum of anatase TiO_2 collected with Bruker D2 Phaser during this study.[13]

Additional excipients that were not listed as an ingredient in the authentic dosage forms were also analyzed. Various carbonates were analyzed to determine if the difference in the cation would produce a different XRD spectrum. In addition to calcium carbonate, magnesium carbonate and sodium carbonate were also analyzed. The spectra of calcium carbonate, magnesium carbonate and sodium carbonate are overlaid and are shown in Figure 8. From these

results, it was found that the different cations present within the compound produced a different XRD spectra, this can be used to differentiate inorganic materials.

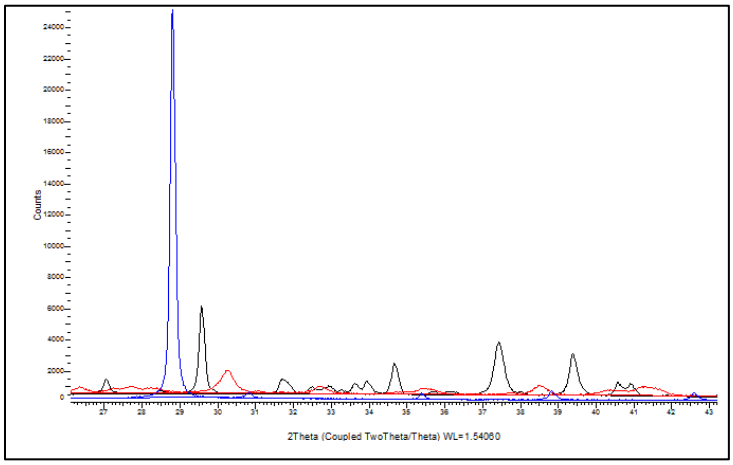

Figure 8. X-ray powder diffraction spectra of various carbonate excipients. Black spectrum is sodium carbonate, blue spectrum is calcium carbonate and red spectrum is magnesium carbonate.

After all excipients and bulk API standards were analyzed, the XRD spectra of the complete authentic dosage forms were collected. The first experiment was to analyze the dosage forms intact, which produced XRD spectra with poor signal to noise ratios. It was found that the coating contributed very little to the spectrum and would not affect the overall pattern; therefore, the authentic dosage forms were then analyzed in the same manner as the excipient and API standards. The only difference in the preparation method was that half of the tablet mass was pressed into a pellet instead of a set amount with the excipients and API standards. A few of the XRD spectra of the authentic dosage forms used in this study can be seen in Figure 9.

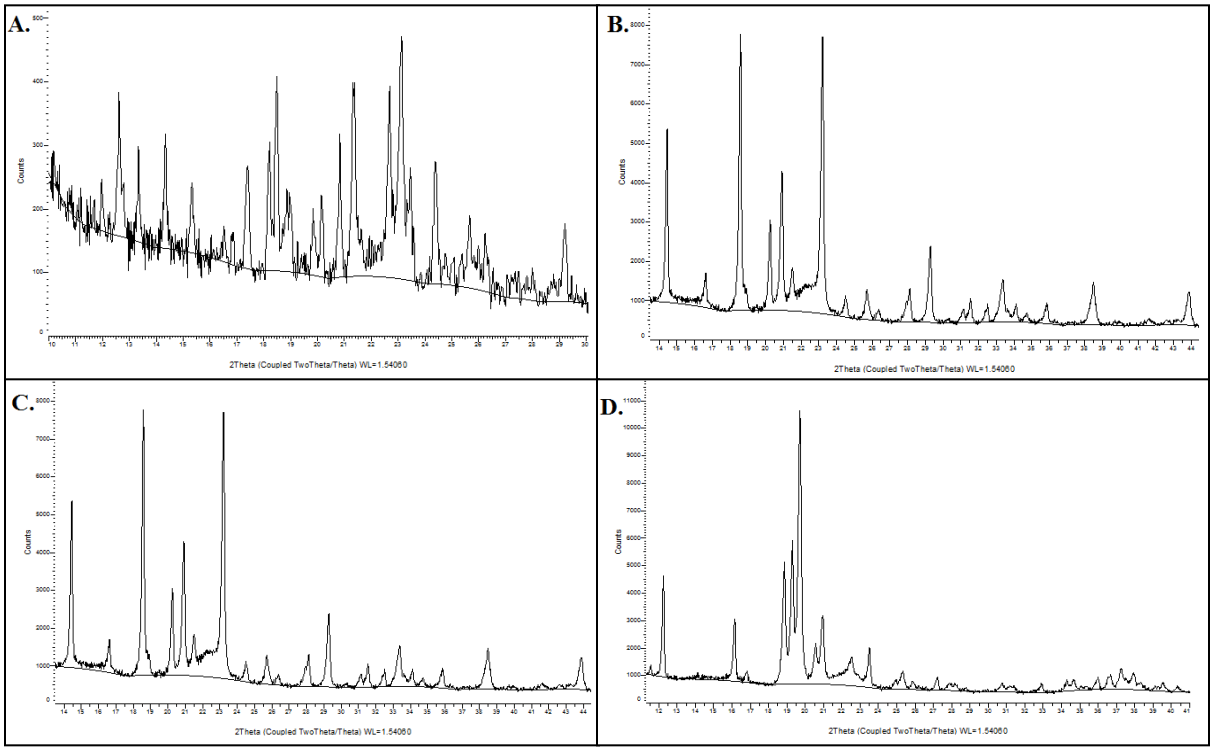

Figure 9. X-ray powder diffraction spectra of only four of the authentic dosage forms in pellets. A. Product 1, B. Product 2, C. Product 3, and D. Product 4.

Reproducibility and variability studies were then conducted. Two pellets were made from one authentic tablet and analyzed to access the variability within a tablet. Then a pellet was created from a separate tablet and analyzed to access the variability between tablets within the same lot. Finally, authentic products from different lots were analyzed and compared to access the variability between lot numbers. The XRD spectra produced from these studies are shown in Figure 10.

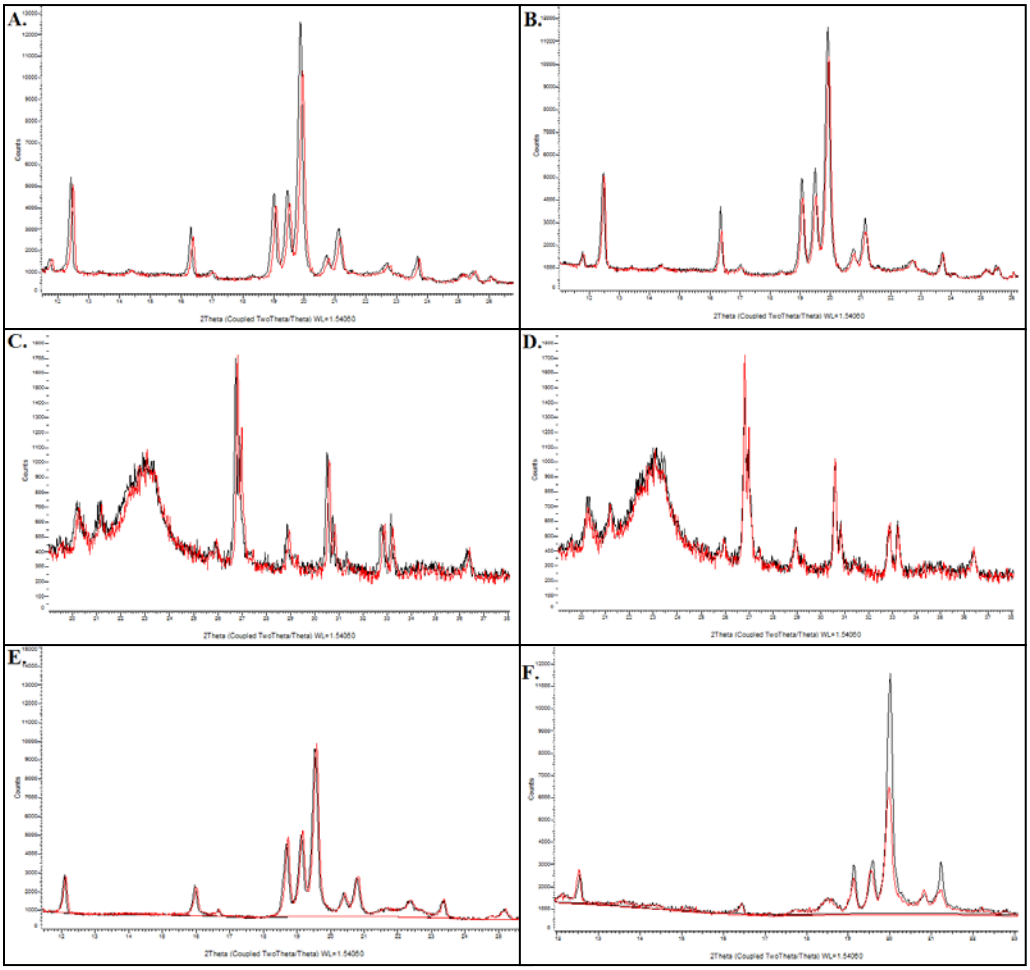

Figure 10. X-ray powder diffraction patterns comparing the variability in one authentic tablet and between two authentic tablets of the same product. A. Two pellets created from the same Product 5 tablet. B. Two pellets created from two Product 5 tablets from the same lot number. C. Two pellets created from the same Product 6 tablet. D. Two pellets created from two Product 6 tablets from the same lot number. E. Product 7 spectra of two pellets from different lot numbers. F. Product 8 spectra of two pellets from different lot numbers.

XRD spectra of authentic dosage forms were then compared to the spectra of excipients and API standards. It was determined to be difficult to assign individual peaks in the dosage form spectra to the bulk powder excipient or API standard spectra. Finally, XRD spectra were collected for known counterfeit dosage forms. Spectra of counterfeit dosage forms were then compared to the authentic product. Several counterfeit XRD spectra with the authentic XRD spectra overlain are shown in Figure 11.

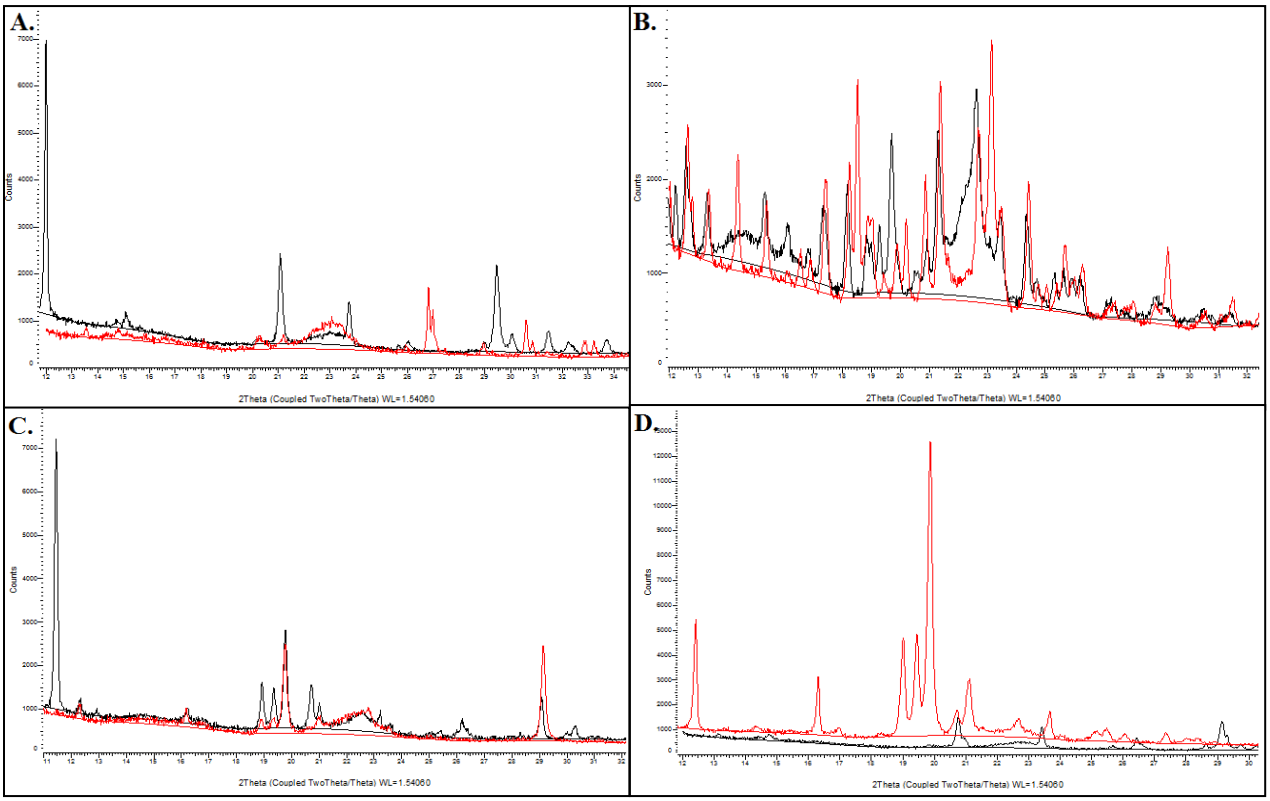

Figure 11. X-ray powder diffraction spectra of four counterfeit and authentic pharmaceutical products. Counterfeit products are in black, while authentic products are in red.

Discussion

It was found that the new Daily Check parameters, established at the beginning of this study, fell within the specifications for the instrument given by the manufacturer. The specifications for 2θ, net height and FWHM and the calculated average compared to the expected value can be seen in Table 4.[4] It is shown that the averages for 2θ at the targeted scan angles fall within the ±0.02° of the expected value, and the averages calculated for the FWHM are all less than the expected $FWHM_{max.}$ The calculated averages for the first two net height values are within the acceptable ±10% of the expected value, but the last two fall outside of the acceptable deviation. This could be due to the increased increment size or decreased scan time of the new parameters. Even though the net height did not fall within the specified limits, the new parameters were considered to be a more practical daily check for this study. Since the method is a qualitative and not quantitative, the critical parameter is peak position and not peak height.

Based on these results, daily performance specifications can be determined and used on a daily basis to evaluate the performance of the instrument.

For method preparations, it was found that adding loose powder to the sample holder was not appropriate because the diffractometer contains cooling fans to keep the instrument at the optimum temperature during analysis. The fans would have caused the powder to blow into the operator's face or distribute sample material within the instrument. The results for the second preparation method, using the mortar and pestle to pack the powder into the sample holder, are shown in Figure 3. From these spectra it can be determined that a larger powder sample yielded a better spectrum. As the amount of powder added to the sample holder increased, the counts or intensity of the different peaks also increased. The results from the third preparation method, using the mortar and pestle to grind the powder then using the Carver press to create a pellet, are shown in Figure 4. It confirmed what was observed in Figure 3, the quality of the XRD spectra was dependent on the amount of product used. With this information it was decided that 60 mg of excipient or API standard was sufficient to produce quality spectra. The two methods were then compared in Figure 5, which indicated that the method using the mortar and pestle and the Carver pellet produced better quality spectra. This can be seen by the defined peak pattern and larger count intensity for each peak. The third method, using the mortar and pestle and Carver press, was then compared to the fourth method, using the Wig-L-Bug device to grind the powder and the Carver press to form a pellet, in Figure 6. It was found that both gave similar results. It can be seen that the spectra that resulted from the mortar and pestle method had slightly higher count values for each of the peaks. It was determined that the Wig-L-Bug device would be used instead, due to ease of use and the consistent powder produced from the device compared to manual grinding.

Bulk excipient standard XRD spectra were collected to later compare with dosage form spectra. The excipients were the first samples to be analyzed with the Bruker D2 Phaser. The spectra were collected and compared to spectra found in literature to confirm that the spectra being produced during this study were correct.[13] A comparison between spectra found in literature and spectra produced during this study of rutile and anatase titanium oxide are shown in Figure 7. Figure 7 shows the XRD spectra produced with the Bruker D2 Phaser gave similar overall spectra with similar peak positions.[13] Differences between the literature references and the spectra collected during this study were the peaks produced by the Bruker D2 Phaser are broader at the base line compared to the reference spectra and differences in peak intensities. This may be due to different instrumentation used and different measurement parameters of the instruments used in analysis. This comparison indicated that the spectra being collected for the various excipients and API standards were accurate representations of the sample.[13]

Additional excipients, such as magnesium carbonate and sodium carbonate, were analyzed to determine if the difference in the cation would produce a distinct XRD spectrum. In Figure 8, the spectra of the three carbonates are overlaid. Each inorganic compound has a distinct XRD spectrum, which could be useful in determining the composition of a counterfeit product. If an unexpected carbonate pattern is observed, the product being analyzed could be considered counterfeit. This is useful in that other analysis methods, such as FT-IR can only distinguish that there is a carbonate present. In addition, and with further investigation, this observation could be applicable to other inorganic compounds, such as silicates, sulfates and phosphates.

Once all excipients and API standards were collected, authentic dosage forms were prepared and analyzed. The XRD spectra of various authentic dosage forms is shown in Figure

9. Each authentic product analyzed produced a distinct spectrum. The reproducibility and variability study results can be seen in Figure 10, the reproducibility studies in Figure 10A and B indicated that the results can be reproduced using the same tablet. It was also shown that there is nominal peak shifts between tablets from the same lot. The XRD spectra from different tablets are consistent with each other. The spectra seen in Figure 10E indicate Product 7 from different lots were consistent with each other, where in Figure 10F there is a difference in the intensity or count value between the lots of Product 8, which could indicate a reformulation of the product between lots, or that during sample preparation the material formed a different orientation in the pellet compared to the first pellet produced. Overall, the authentic products did produce similar XRD spectra between tablets and lots.

Within these studies, a slight shift in peaks was observed, but according to USP general chapters<941> peak shifts are common occurrences when analyzing powder material. It was mentioned that peak shifts less than 0.2° at a 2θ angle position indicates the sample is considered the same crystalline form and therefore the same product. Table 5 contains the peak position over a selected range of the various variability studies. From this table it can be concluded that all pellets produced similarXRD spectra because no peak shifts above 0.2° were observed.

Table 5. Peak positions from the various variability studies at select peak positions.

Same Tablet		Different Tablets		Different Lot Numbers	
Pellet 1 Peak Positions (2θ)	Pellet 2 Peak Positions (2θ)	Tablet 1 Peak Positions (2θ)	Tablet 2 Peak Positions (2θ)	Lot # 1 Peak Positions (2θ)	Lot # 2 Peak Positions (2θ)
12.424	12.500	20.232	20.271	12.095	12.146
16.306	16.395	26.813	26.813	15.962	16.025
19.016	19.079	26.813	26.994	18.709	18.749
19.458	19.521	28.914	28.954	19.138	19.184
19.874	19.949	30.594	30.600	19.549	19.596

The authentic dosage form XRD spectra were then collected and overlaid with their corresponding excipients and API standards. It was difficult to visually distinguish the API within the authentic dosage form XRD spectrum. This may be due to the manipulation of the API during the manufacturing process. The API may change crystalline structure during this process, resulting in a variation from the bulk API standard. This could cause the XRD spectra comparison of the bulk API standard to the API within the dosage form to be difficult; both would produce different spectra depending on the crystalline structure it possessed. The low concentration of the API within the dosage form by weight percent may also be below the detection limits of the XRD instrumentation.[13] If this were the case, the API concentration would be too low for the XRD instrumentation to detect and give results. Based on potential changes in crystallinity of the API during the manufacturing process and API concentration, the observation of the absence of the API, presence of additional APIs, or the presence of a different API within a counterfeit product should not be used as criteria for determining authenticity of a product using XRD spectral analysis.

Unlike the APIs, various excipients can be observed within the authentic XRD spectrum. In Figure 10E, between 18.3° and 21.5° an observable pattern indicative of lactose monohydrate (pink spectrum) can be seen within the authentic dosage form (black spectrum). The peak shift observed between the spectra is caused by the difference in the crystalline form of the lactose monohydrate located within the dosage form and the bulk excipient powder. According to the United States Pharmacopeia (USP) general chapter <941>, peak shifts greater than 0.2° at a given 2θ-diffraction angle are indicative of a different crystalline structure. Counterfeit pharmaceuticals contain various excipients with different crystalline structures than those found within the authentic product. With these parameters, a suspect product that produces an XRD

spectrum with peak shifts great than 0.2° at a given 2θ-diffraction angle would be considered a suspect counterfeit product.

Known counterfeit dosage forms were then analyzed and compared to the corresponding authentic dosage forms. A few of the spectra with overlaid authentic spectra can be seen in Figure 11. By comparing the authentic and counterfeit spectra, Figure 11A shows the counterfeit spectrum contains various additional peaks; while Figure 11D shows the counterfeit product is missing various peaks found within the authentic product. Therefore, in the samples analyzed as part of this project, counterfeit XRD spectra will be missing peaks, have additional peaks, or have similar peaks with a peak shift of greater than 0.2° at a given 2θ-diffraction angle. The counterfeit spectrum in Figure 12C does not have a peak shift, meaning the peaks that overlap with the authentic product are likely to have similar crystalline structures, but the spectrum is missing various peaks indicating the product is counterfeit.

Conclusion

In conclusion, a method was developed to analyze and produce quality XRD spectra of pharmaceutical products, authentic, suspect, or counterfeit, using the Bruker D2 Phaser x-ray powder diffractometer. The best method preparation for analysis was to use the Wig-L-Bug device to finely ground the powder and the Carver press to create a pellet. About 60mg of bulk excipient and API standards were necessary for analysis, while half the tablet mass was needed for the dosage forms. It was found that authentic dosage form products result in the same overall XRD spectra between tablets and lot numbers, with the exception of peak shifts less than 0.2º. Peak shifting of less than 0.2° is a normal occurrence when analyzing powder material, the shift is due to the random orientations of the crystalline material during the packing process, each sample will possess different crystalline orientations.[14]

The method was validated with the use of information found in USP general chapter <941> stating that if peak shifts within a suspect product XRD spectrum are greater than 0.2º for a given 2θ-diffraction angle compared to the authentic XRD spectrum, then the product meets the criteria for a counterfeit product. Counterfeit pharmaceuticals can also be distinguished from authentic pharmaceuticals by examining the overall XRD spectra. Counterfeit product spectra will contain missing peaks, additional peaks and peak shifts. This method and validation will allow the Forensic Chemistry Center to use the diffractometer as an additional technique to distinguish counterfeit pharmaceuticals from authentic pharmaceuticals.

Future Work

Additional studies should be conducted to determine a shorter daily check scan that could be performed before each use of the instrumentation. Various setting manipulations and statistical evaluation of the new parameters would need to be conducted to determine that the scan fell within specified limits. Currently, spectra are being overlaid and visually compared to determine authenticity; a future project would be to build a user spectral library with authentic and counterfeit dosage form products, excipients and APIs. With the library built, the peak searching capabilities on the software could be used to easily give a spectral match to the analyzed product. In addition, more reproducibility studies could be conducted to determine if the same results can be achieved by different analysts. Different analysts would need to be trained using the developed method, analyze the product and then compare the results to the already collected spectra and each other's spectra. Finally, it would be beneficial to determine a method to identify the APIs within the dosage forms. A proposed method was to extract and recrystallize the API from the dosage form and then compare the spectrum to the API standard.

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Disclosure

The mention of specific products/instruments in this presentation is for information purposes only and does not constitute an endorsement by the Food and Drug Administration and/or the Forensic Chemistry Center.

Appendix 1

API
Aripiprazole
Risedronate sodium
Tadalafil
Losartan potassium
Hydrochlorothiazide
Atorvastatin
Clopidogrel Bisulfate
Clopidogrel Hydrogen Sulfate Form I
Clopidogrel Hydrogen Sulfate Form II
Atazanavir sulfate
Montelukast sodium
Abacavir sulfate
Lamivudine
Zidovudine
Sildenafil citrate
Alprazolam
Ezetimibe
Olanzapine

Excipients
cornstarch
lactose monohydrate
magnesium stearate
microcrystalline cellulose
Crospovidone
hydroxypropyl methylcellulose
polyethylene glycol
titanium dioxide
croscarmellose sodium
hypromellose
sodium lauryl sulfate
talc
starch soluble
calcium carbonate
candelilla wax
mannitol
anhydrous dibasic calcium phosphate
cellulose
docusate sodium
sodium benzoate
carnauba wax