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Identification of drugs in pharmaceutical dosage forms by X-ray powder diffractometry

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Abstract

A simple X-ray powder diffractometric (XRD) method was developed for the identification of the active ingredient in a variety of dosage forms. The method was successfully used to unambiguously identify the active ingredient(s) in tablet, capsule, suppository and ointment formulations. The unique feature of the method is that it provides information about the solid-state of the drug. Thus, a capsule formulation containing anhydrous ampicillin was readily distinguished from that containing ampicillin trihydrate. The USP stipulates the use of the β-polymorphic form of anhydrous carbamazepine in carbamazepine tablets. Contamination by the α-polymorph (down to a level of 1.4% w/w of the formulation) could be detected. In some of the multicomponent formulations, there was a pronounced overlap of the powder patterns of ingredients which made identification difficult. This problem was solved by using a pattern subtraction technique, which permitted selective subtraction of the XRD pattern of the constituents of the formulation from the overall XRD pattern. Such an approach enabled identification of the drug even when it constituted only 5% w/w of the formulation. The method also permitted simultaneous identification of the multiple active ingredients in trimethoprim-sulfamethoxazole and acetaminophen-aspirin-caffeine formulations.

Keywords: Identification; X-ray powder diffractometry; Pattern subtraction; International Centre for Diffraction Data; Solid-state

1. Introduction

The monograph of every compound listed in the United States Pharmacopeia (USP) contains one or more identification tests [1]. Many compendial substances are identified on the basis of a test for a functional group in the molecule. IR spectrophotometry is also a widely recommended method, wherein the spectrum of the test compound is compared with that obtained concomitantly of the USP Reference Standard. While the monographs of dosage forms also contain identification tests for the active ingredient, these tests are complicated by the presence of excipients in the formulation. This often necessitates extraction of the active ingredient from the dosage form.

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There are several shortcomings in the currently available identification tests. The sample is not analyzed directly and needs pretreatment. In addition to being labor intensive, these procedures can bring about alterations in the properties of the substance being identified. For example, the IR method usually requires intimate mixing of the test substance with potassium bromide which is then compressed into a pellet and analyzed. Alterations in solid-state can be brought about by compression [2]. Compression can be avoided and IR spectra obtained by finely grinding a solid and dispersing it in mineral oil [3]. It is now recognized that grinding for even as short a time period as 5 s, can bring about alterations in solid-state [4]. When a functional group in the molecule forms the basis for identification, the test tends to be nonspecific.

The pharmacopeial identification tests of the drug in a dosage form tend to be more complicated than the analysis of the drug alone. If the identification procedure requires extraction of the active ingredient (which is usually the case), then potentially valuable information about the solid-state of the drug is lost. This can also be a serious disadvantage of the identification test.

The oral route continues to be extensively used for administration of drugs. The most popular dosage forms are tablets and capsules (hard gelatin) wherein the drug is usually present as a solid. It is becoming increasingly clear that solid-state properties (polymorphic form, state of solvation, degree of crystallinity) of the active ingredient can profoundly influence the in vivo performance of the dosage form [5]. Unfortunately, most of the identification methods currently listed in the USP are insensitive to the solid-state of the drug in the dosage form.

X-ray powder diffractometry (XRD) is a powerful technique for the identification of crystalline solid phases [6]. Every crystalline solid phase has a unique XRD pattern which can form the basis for its identification. In a powder mixture, each crystalline phase produces its pattern independently of the other constituents in the mixture [7]. The technique is unique, since it combines absolute specificity with a high degree of accuracy [8]. Despite these attributes, the method finds very limited application for the evaluation of drug product quality [1].

The aim of this project was to develop XRD methods for the identification of the active ingredient in a variety of pharmaceutical dosage forms. The methods were simple and in most cases, the dosage forms were analyzed directly with minimal or no sample pretreatment. Since there was no need to extract the active ingredient from the dosage form, the identification was accomplished in presence of the excipient(s). The unique advantage of the method was that it provided information about the solid-state of the active ingredient. While the specific polymorphic form was unambiguously identified, the distinction between the solvated and unsolvated (anhydrous) forms of a compound was possible, when the crystal lattices of the two forms were different. The technique permitted simultaneous identification of more than one active ingredient in formulations. Based on studies with model formulations, drug identification was possible even when the weight fraction of the active ingredient was as low as 0.05.

2. Materials and methods

2.1. Materials

Acetaminophen, anhydrous ampicillin ($C_{16}H_{19}N_3O_3S$), ampicillin trihydrate ($C_{16}H_{19}N_3O_3S \cdot 3H_2O$), caffeine, anhydrous β-carbamazepine, chlordiazepoxide hydrochloride, sulfamethoxazole, trimethoprim (all from Sigma Chemical, St. Louis, MO), zinc oxide, acetyl salicylic acid (J.T. Baker Chemical, Phillipsburg, NJ), corn starch (Pure-Dent™, Grain Processing, Muscatine, IA), hydroxypropyl cellulose (Klucel type EF, Hercules, Wilmington, DE), hydroxypropyl methylcellulose (Methocel K15M, Dow Chemical, Midland, MI), microcrystalline cellulose NF (Avicel® PH 105 and Avicel® PH 101, FMC, Philadelphia, PA), sodium starch glycolate, NF (Explotab®, Edward Mendell, Patterson, NY) and fumed silicon dioxide (Cab-O-Sil®, Cabot, Tuscola, IL) were used as received. Magnesium stearate NF (Fisher Scientific, Fair Lawn, FN) and microcrystalline cellulose NF (Avicel® PH

102, FMC, Philadelphia, PA) were passed through a 100 mesh sieve before use. α-Carbamazepine was prepared from β-carbamazepine according to the procedure described earlier [9].

Anhydrous ampicillin capsules (Omnipen®, Wyeth Labs., Philadelphia, PA), ampicillin trihydrate capsules (Principen®, E.R. Squibb and Sons, Princeton, NJ), chlordiazepoxide hydrochloride capsules (Halsey Drug, Brooklyn, NY), acetaminophen caplets (Tylenol® caplets, McNeil, Fort Washington, PA), sulfamethoxazole-trimethoprim tablets (Bactrim™, Roche, Nutley, NJ) aspirin-acetaminophen-caffeine tablets (Excedrin® extra strength, Bristol-Myers Squibb, New York, NY) and zinc oxide ointment (Walgreens, Deerfield, IL) were locally purchased. Acetaminophen suppositories (Uniserts®, Upsher-Smith, Minneapolis, MN) and placebo suppositories were gifts from Upsher-Smith Laboratories.

2.2. Sample preparation for powder X-ray diffractometry

2.2.1. Powders

The sample holder was made of aluminum and consisted of a square central cavity, 15 mm × 15 mm × 1.5 mm. This cavity extended to one side of the holder and this channel was used to fill the powder into the holder. Unless otherwise noted, the powder was filled into the holder by the side drift technique [10]. A glass slide was clipped up to the top face of the sample holder first, so as to form a wall. The holder was then held in a vertical position and about 30 mg of powder was poured into the cavity via the open channel. The holder was then tapped gently, for a preset number of times. This procedure was repeated until the cavity was filled. The channel was closed with an aluminum plate which was held in position by a screw. Finally, the glass slide was carefully removed without disturbing the sample surface. Powder outside the square central cavity, if any, was removed.

2.2.2. Tablets

The tablets were gently ground into a fine powder using a glass mortar and pestle.

2.2.3. Capsules

The powder was removed from the gelatin shell. The contents of 2 or 3 capsules were combined and filled into the sample holder.

2.2.4. Ointment

The ointment was filled into the cavity of the sample holder from the top. A glass slide was used to make the surface smooth.

2.2.5. Suppositories

The suppositories were cut into very thin pieces with a razor blade and filled into the cavity of the sample holder from the top.

2.2.6. Analysis of intact tablets

The sample holder was made of aluminum and consisted of a circular central cavity, 11.3 mm in diameter and 2.3 mm deep [11]. Two small pieces of molding clay were put at the bottom of the holder, the tablet was dropped into the cavity, and using a flat glass slide, the tablet was gently pressed down until the holder surface and the glass surface were coplanar.

2.3. Powder X-ray diffractometry

Samples were exposed to CuKα radiation (45 kV × 30 mA) in a wide-angle powder X-ray diffractometer (Model D500, Siemens). The Bragg-Brentano focusing geometry was used, with a 1° incident aperture slit, a 0.15° detector slit, and a scintillation counter as the detector. Unless otherwise stated, the instrument was operated in the step-scan mode in increments of 0.05°2θ, and counts were accumulated for 1 s at each step.

3. Results and discussion

3.1. Availability of reference patterns

It is well known that X-ray powder diffractometry (XRD) is a powerful technique for the identification of crystalline solid phases. An added advantage is that the reference diffraction patterns of numerous compounds are readily available. The International Centre for Diffraction

Data (ICDD) maintains a collection of single-phase XRD patterns [12]. ICDD also assigns a quality mark, based on strict evaluation criteria, to each pattern in the database. A '*' mark indicates data of the highest quality and to qualify for this mark, the compound must have a well characterized chemical composition. The intensities of the X-ray lines must be measured objectively and instrumentally and no serious systematic errors may occur. Lines with d-spacings ≤ 2.50 Å must retain at least three significant digits after the decimal point. To qualify for the 'i' mark, there can be a maximum of two unindexed or impurity lines, provided none of these belong to the strongest eight. If the data is of low precision, or if the data is due to a poorly characterized or multiphase system, an 'o' mark is assigned. Patterns that do not meet any of the above mentioned quality marks are left blank. Extensive details about the quality mark can be found in ICDD publications [12]. There are separate listings of inorganic, mineral and organic compounds.

In the ICDD publications, the d-spacings (interplanar spacings) of the X-ray lines (in Å) and their relative intensities are tabulated. Once an XRD pattern is experimentally obtained, it can be compared with the pattern published by ICDD. It is therefore possible to objectively compare an experimentally obtained XRD pattern with that reported in the database on a line by line basis. If the ICDD data of a test compound is of high quality ('*' or 'i' mark), and the experimentally observed XRD pattern shows excellent agreement with it, then there is unambiguous identification of the compound.

3.2. Identification of drug present in different types of dosage forms

Identification of the drug compound was extremely simple. The XRD pattern of the test compound was experimentally obtained. This was then compared with the XRD pattern of the compound published by the ICDD. Identification of the active ingredient in a dosage form required an additional step. The XRD pattern of the dosage form was experimentally obtained. These two XRD patterns were compared with that of the active ingredient reported in the ICDD.

The experimentally obtained XRD data of acetaminophen powder and an acetaminophen tablet formulation (Tylenol® caplet) are presented in Table 1. The table also contains the card pattern published by the ICDD [13], which is data of the highest quality ('*' mark). A comparison of the XRD pattern of acetaminophen obtained in our laboratory with the ICDD pattern reveals in general, a good agreement of the line positions (d-spacings of the lines).

In the reference pattern, three lines with very close d-spacings of 3.65, 3.62 and 3.60 Å (2θ values of 24.36, 24.57 and 24.70°, respectively) are reported (Table 1). One of these lines (3.62 Å) is missing in the acetaminophen XRD pattern obtained in our laboratory. The instrument is unable to resolve the lines with d-spacings of 3.62 and 3.60 Å and therefore we observe a single line with a d-spacing of 3.60 Å. Since the missing line is in close proximity to other lines that have been detected, the issue is not of serious concern. The resolution of closely spaced lines can be influenced by both the nature of the sample and by instrumental factors. The width of the X-ray lines (full width at half maximum) is dependent on the sample particle size as well as its crystallinity [14–16]. Any sample induced line broadening will make it difficult to distinguish between lines with close d-spacing values. There are also several instrumental factors that influence peak resolution [14].

A comparison of the relative intensities of the X-ray lines does not reveal a good agreement. The intensity of the X-ray lines can be affected by several factors including preferred orientation. A microscopic examination of the acetaminophen powder revealed that about 80% of the particles were acicular and the rest of the particles were plate-like. A majority of the particles were less than 50 μm in size (the longest dimension). However, a significant number of particles were also larger than 50 μm and particles as large as 500 μm were observed. We could have minimized preferred orientation by grinding the particles. However, milling was avoided for the following reasons. (i) Milling can induce phase transitions in

Table 1
XRD data of acetaminophen powder and a marketed acetaminophen tablet formulation

ICDD card pattern of acetaminophen (13)		Acetaminophen powder		Acetaminophen[b] tablet	
d-spacing (Å)	Relative intensity[a] (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)
7.30	29	7.34	13	7.34	21
6.41	37	6.44	23	6.44	31
5.81	47	5.81	24		
5.71	71	5.73	36	5.73	54
5.29	21	5.32	12	5.32	17
4.94	34	4.98	8		
4.87	56	4.88	100	4.90	53
4.35	21	4.37	14	4.37	24
4.27	15	4.29	13	4.29	12
3.85	16	3.86	21	3.87	11
3.78	59	3.80	34	3.80	66
3.65	100	3.66	51	3.67	80
3.62	10				
3.60	8	3.60	9	3.60	8
3.36	77	3.37	56	3.37	100
3.30	9	3.29	16	3.29	20
3.28	15	3.28	17		
3.08	7	3.09	5	3.08	8
3.05	6	3.06	9	3.06	8
2.75	9	2.76	8	2.76	10
2.74	9	2.74	10	2.74	10
2.73	7	2.72	5		
2.48	9	2.48	15	2.48	9
2.44	9	2.44	8	2.44	11
2.43	8	2.43	7		
2.40	7	2.40	7	2.41	8
2.34	6	2.34	3	2.34	5

[a]Only lines with relative intensities >5% are considered here.
[b]Tylenol® caplet.

solids [17]. (ii) We wanted to keep the method as simple as possible and the simplest method is direct analysis of the powder with no pretreatment whatsoever. (iii) Since the goal of the project is phase identification, our predominant interest is the position of the X-ray lines (d-spacings). Therefore, from now on, the discussion will be restricted to the d-spacings of the X-ray lines. However, for the sake of completeness, the relative intensities of the lines are also provided.

The high intensity lines that characterize acetaminophen are all observed in the powder pattern of the acetaminophen tablet formulation (Tylenol® caplet). Thus, XRD permits ready identification of the active ingredient in the dosage form.

In the acetaminophen tablet, the weight fraction of drug was determined to 0.83. The formulation contains cellulose, hydroxypropyl methylcellulose, magnesium stearate, starch and sodium starch glycolate as excipients [18]. No X-ray lines due to excipients were observed in the XRD pattern of powdered acetaminophen tablets. This could be attributed to the poorly crystalline nature of the excipients under consideration and/or their low weight fraction in the formulation. Some X-ray lines of acetaminophen (lines with d-spacings of 5.81, 4.94, 3.28, 2.73 and 2.43 Å) are absent. Two of these are low intensity lines (d-spacings of 2.73 and 2.43 Å) whose absence can be explained as being due to the dilution of the

drug in the formulation. The lines with d-spacings of 5.81, 4.94 and 3.28 Å are closely spaced to lines with d-spacings of 5.71, 4.87 and 3.30 Å, respectively. In these instances, the instrument appears to be unable to resolve lines with close d-spacings.

The identification of acetaminophen in the tablet formulation was simplified by the fact that the observed X-ray lines were only due to the active ingredient. The next object was to identify acetaminophen in a dosage form wherein at least one excipient exhibited a characteristic diffraction pattern. Acetaminophen suppositories were selected for these studies.

In addition to acetaminophen, these suppositories contain hydrogenated vegetable oil, polyoxyethylene stearate, glycerol monostearate, and preservatives. A comparison of the XRD patter of acetaminophen (Fig. 1a) with that of acetaminophen suppository (Fig. 1b) reveals marked differences in the angular range of 19–25°2θ. Fig. 1c is the XRD pattern of the placebo suppository. It is clear that the X-ray lines of the suppository base interfere with those of acetaminophen. In order to identify the drug, it was necessary to remove the contribution made by the base to the overall diffraction pattern (Fig. 1b). By using a pattern subtraction technique we attempted to selectively remove the contribution of the suppository base to the XRD pattern of the formulation [19].

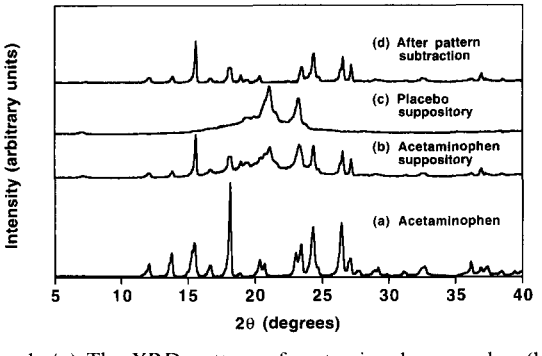

Fig. 1. (a) The XRD pattern of acetaminophen powder. (b) The XRD pattern of acetaminophen suppository. (c) The XRD pattern of a placebo suppository. (d) The residual XRD pattern after proportional subtraction of the placebo suppository pattern from the acetaminophen suppository pattern.

In order to perform this pattern subtraction, it was necessary to know the weight fraction of the crystalline active ingredient in the formulation. Based on the weight of each suppository and the acetaminophen content in each suppository, the acetaminophen weight fraction was determined. However, the weight fraction of crystalline drug will be less if some of the drug is dissolved in the suppository base [20,21]. The crystalline acetaminophen content was estimated by DSC. The enthalpy of fusion of pure crystalline acetaminophen was determined to 6.8 kcal $\times$ mol^{-1} (melting point 170.6°C). This value was identical to that reported in the literature [22]. The acetaminophen suppository exhibited an endotherm at 168.3°C (due to the crystalline acetaminophen) with an enthalpy value of 1.6 kcal $\times$ mol^{-1} from which the weight fraction of crystalline acetaminophen was estimated to be 0.24. This was less than the calculated acetaminophen weight fraction of 0.31 (labeled acetaminophen content per suppository/weight of each suppository). If the matrix is defined to consist of everything in the formulation except the crystalline acetaminophen, the matrix weight fraction is 0.76. Using this value, the XRD pattern of the placebo (Fig. 1c) was subtracted from that of the acetaminophen suppository (Fig. 1a). The d-spacings of the lines observed in the background subtracted pattern (Fig. 1d) showed an excellent agreement with those of the acetaminophen lines (Fig. 1a). Therefore, pattern subtraction appears to be a viable technique for identification of the active ingredient in a complex formulation.

3.3. Identification of drug present in different states of hydration

Many drugs are listed in the USP both in an anhydrous form and as a hydrate wherein water is incorporated, usually stoichiometrically, into the crystal lattice. Some examples are ampicillin, caffeine, prednisolone and theophylline [1]. The anhydrous and hydrate forms can exhibit pronounced differences in pharmaceutical properties such as dissolution rate, powder flow and bioavailability [23,24].

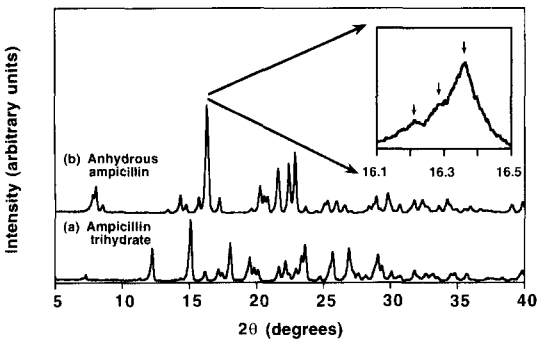

Fig. 2. (a) The XRD pattern of ampicillin trihydrate powder. (b) The XRD pattern of anhydrous ampicillin powder. Inset shows an expanded view of the diffraction pattern of anhydrous ampicillin in the region 16.1–16.5°2θ, in order to show details of the overlapped peaks.

Based on crystal lattice studies, there are three possible situations when a hydrate is dehydrated [25].

(i) The crystal lattice of the anhydrate is nearly identical to that of the original hydrate. In this case, the XRD patterns of the hydrate and the anhydrate will be similar.

(ii) The residue does not exhibit long range lattice order and therefore, the anhydrate formed will exhibit a poorly defined XRD pattern.

(iii) The anhydrate recrystallizes with a different crystal lattice. In this case, the XRD patterns of the hydrate and the anhydrate will be different and these differences can be exploited for identification purposes. Fortunately, many compounds of pharmaceutical interest belong to this category.

In this study, ampicillin was used as the model compound. The USP lists the anhydrous ($C_{16}H_{19}N_3O_3S$) and trihydrate ($C_{16}H_{19}N_3O_3S \cdot 3H_2O$) forms of ampicillin [1]. Marketed ampicillin capsules contain either the anhydrate or the trihydrate [18,26], and these two forms exhibit pronounced differences in their XRD patterns (Fig. 2). A comparison of the two XRD patterns reveals that, in the angular range where some peaks of anhydrous ampicillin occur, there are no peaks of ampicillin trihydrate and vice versa [27,28]. For example, lines with d-spacings of 10.89 Å (8.11°2θ), 5.63 Å (15.73°2θ), 5.39 Å (16.43°2θ) and 4.25 Å (20.88°2θ) were unique to anhydrous ampicillin and lines with d-spacings of 7.19 Å (12.30°2θ), 5.86 Å (15.11°2θ), 3.75 Å (23.71°2θ), and 3.46 Å (25.73°2θ) were unique to ampicillin trihydrate. Thus XRD has the potential to identify the state of hydration of the drug in the formulation.

The first effort was directed towards identification of anhydrous ampicillin in a capsule formulation. Before the analysis of the formulation, the XRD pattern of anhydrous ampicillin was experimentally obtained and compared with the ICDD card pattern [27]. There was in general, a good agreement between the two (Table 2). However, several lines were missing in the XRD pattern obtained in our laboratory. The d-spacings of the missed lines were close to the d-spacings of other lines that have been detected. As with acetaminophen, this appeared to be an issue of resolution of closely spaced lines.

Better resolution was achieved in an instrument with a wider focusing circle and a solid-state detector (Scintag XDS2000). The data was collected in the continuous scan mode over the angular range of 16.10–16.50°2θ. The most intense line of anhydrous ampicillin occurs in this angular range. After smoothing of the data (using the software DMS2000, version 3.14), three lines with d-spacings of 5.46, 5.44 and 5.41 Å were detected (Fig. 2, insert). Therefore, missing lines could be detected by improving the instrumental resolution. However, any adjustment of instrumental factors to increase resolution will have a detrimental effect on the line intensities. For routine XRD work, the instrumental settings usually reflect a compromise between maximum resolution and maximum intensity.

A capsule formulation containing anhydrous ampicillin (Omnipen®) was selected and in order to keep the analysis simple, XRD of the intact capsule was attempted [18]. This approach had to be abandoned since the powder pattern consisted of an amorphous halo (between 10 and 30°2θ), due to the gelatin shell.

The powder was removed from the capsule shell and subjected to XRD. The XRD patterns of ampicillin capsules and ampicillin powder show excellent agreement (Table 2). Anhydrous ampicillin constituted approximately 83% w/w of the powder in each capsule. The excipients in the

Table 2
XRD data of anhydrous ampicillin powder and a marketed anhydrous ampicillin containing capsule formulation

ICDD card pattern of ampicillin (27)		Ampicillin powder		Ampicillin capsules[b]	
d-spacing (Å)	Relative intensity[a] (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)
11.24	30	11.33	19	11.33	12
10.89	90	10.97	27	10.97	24
10.26	8	10.33	8	10.33	4
6.58	8	6.63	3	6.63	2
6.16	14	6.19	19	6.19	9
5.98	12	6.00	9	6.00	7
5.63	25	5.64	17	5.64	12
5.45	20				
5.42	40				
5.39	100	5.34[c]	100	5.42[c]	100
5.31	10				
5.13	8	5.15	16	5.15	8
4.51	12	4.54	3	4.55	4
				4.45	8
4.37	45	4.39	25	4.38	24
4.31	25	4.32	18	4.32	12
4.25	30	4.27	17	4.27	14
4.09	55	4.11	48	4.11	35
3.96	25	3.97	53	3.97	31
3.88	35	3.89	65	3.89	41
3.75	10	3.77	6	3.76	6
3.54	16	3.56	10	3.55	9
3.51	25	3.52	14	3.52	13
3.43	12	3.43	13	3.43	12
3.35	12	3.36	9	3.36	8
3.13	12	3.15	7	3.14	5
3.10	14	3.11	6	3.09	8
3.08	10	3.08	17		
2.99	16	3.00	21	3.00	15
2.97	20				
2.91	20	2.92	8	2.91	7
2.82	8				
2.81	25	2.82	14	2.82	10
2.77	8				
2.76	14	2.77	11	2.77	11
2.74	6				
2.72	10	2.73	7	2.73	5
2.66	12	2.67	7	2.66	5
2.62	10	2.62	14		
2.60	16	2.60	9	2.62	10
2.56	8	2.57	4	2.57	3
2.52	12	2.53	4	2.52	3
2.49	14	2.50	6	2.50	5
2.44	8	2.45	3	2.45	3
2.31	8	2.31	9	2.31	7
2.30	8				
2.26	6	2.26	12	2.26	8

[a]Only lines with relative intensities >5% are considered here.
[b]Omnipen®.
[c]This is actually a composite of three overlapping lines. See insert in Fig. 2.

formulation were lactose, methylcellulose, stearic acid and titanium dioxide [18]. The XRD pattern of ampicillin capsules revealed an extra line at 4.45 Å which is likely due to an excipient (Table 2). α-lactose monohydrate is characterized by an intense line with a d-spacing of 4.46 Å [29]. Therefore, the line with a d-spacing of 4.45 Å could be attributed to lactose. Thus XRD has the potential ability to identify not only the active ingredient, but also the crystalline excipients in a formulation.

Using the same experimental approach, we were successful in identifying ampicillin trihydrate in a capsule formulation. First the XRD pattern of ampicillin trihydrate was experimentally obtained and compared with the ICDD card pattern [28]. The two patterns showed good agreement. Next, the powder removed from ampicillin trihydrate capsules (Principen®) [26] was subjected to XRD. The XRD pattern of the ampicillin trihydrate capsules was identical to that of ampicillin trihydrate.

3.4. Identification of drug when it constitutes a small weight fraction of the formulation

We have so far demonstrated that the crystalline active ingredient can be readily identified so long as it forms a substantial fraction of the formulation. However, in many cases, the drug constitutes a small weight fraction of the solid formulation. Some examples are the cardiac glycosides (digoxin, digitoxin), benzodiazepines (chlordiazepoxide, diazepam) and steroids (estradiol, norethindrone). In these instances, XRD may lack the sensitivity to detect the active ingredient.

Using chlordiazepoxide hydrochloride (hereafter referred to as chlordiazepoxide) as the model compound, the sensitivity of XRD was evaluated. Chlordiazepoxide is commercially available as a capsule formulation and the drug content can range from 5–25 mg per capsule [18].

Mixtures containing different weight fractions of chlordiazepoxide and microcrystalline cellulose were prepared. While chlordiazepoxide has a characteristic XRD pattern (Fig. 3a), microcrystalline cellulose exhibits a broad amorphous halo (Fig. 3c). Identifying chlordiazepoxide was no problem so long as its weight fraction was ≥ 0.10. When the drug weight fraction was decreased to 0.05, its presence was not readily discernible (Fig. 3b). Using the pattern subtraction technique discussed earlier, the XRD pattern of microcrystalline cellulose was subtracted from the XRD pattern of the drug-microcrystalline cellulose mixture (Fig. 3d). This permitted ready identification of chlordiazepoxide (compare Fig. 3d with 3a).

3.5. Identification of drug in an ointment formulation

Ointments are semisolid products designed for external application to the body [30]. The physical state of the drug depends on the solubility of the drug in the ointment base. The two possibilities are, (a) the drug is dissolved in the base, or (b) a fraction of the incorporated drug is dissolved and the rest is dispersed in the matrix. If the latter situation exists, XRD is potentially useful to identify the drug in the formulation.

First, the XRD pattern of zinc oxide powder was obtained (Fig. 4a), followed by that of zinc oxide ointment (Fig. 4b). The two patterns were similar. The powder pattern of zinc oxide ointment exhibited 3 lines with d-spacings of 2.82 (peak at 31.70°2θ), 2.61 (34.35°2θ) and 2.48 Å (36.20°2θ). These were in excellent agreement with the reported d-spacings of 2.81, 2.60 and

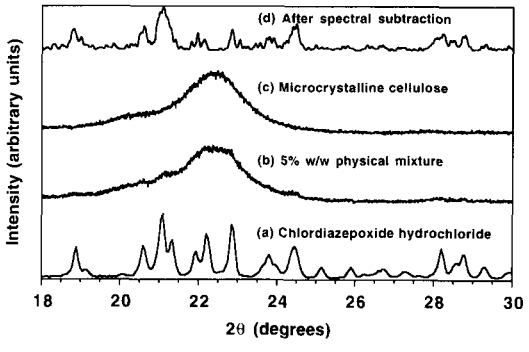

Fig. 3. (a) The XRD pattern of chlordiazepoxide hydrochloride powder. (b) The XRD pattern of a powder mixture of chlordiazepoxide hydrochloride (5% w/w) and microcrystalline cellulose (95% w/w). (c) The XRD pattern of microcrystalline cellulose powder. (d) The residual XRD pattern after proportional subtraction of the microcrystalline cellulose XRD pattern from that of the physical mixture (b). The full scale in this case is different from that of the other three XRD patterns.

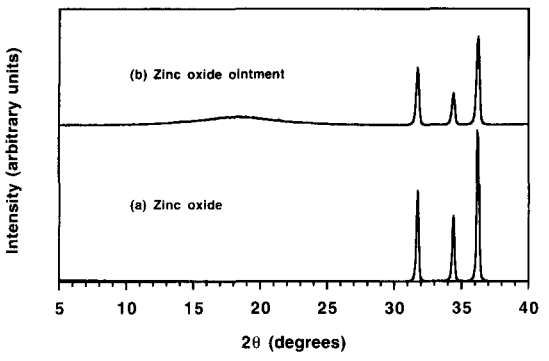

Fig. 4. (a) The XRD pattern of zinc oxide powder. (b) The XRD pattern of a 20% w/w zinc ozide ointment.

2.48 Å, respectively [31]. The ointment also exhibited a broad amorphous halo between 10 and 25°2θ which could be attributed to the amorphous character of the ointment base.

3.6. Identification of an undesirable polymorph

The β-polymorphic form of anhydrous carbamazepine is official in the USP [1]. The USP monograph of carbamazepine stipulates that, 'The X-ray diffraction pattern conforms to that of USP Carbamazepine Reference Standard, similarly determined'. Interestingly, no limits have been set in the USP for the other polymorphs of anhydrous carbamazepine. Although there are reports of several polymorphic forms of anhydrous carbamazepine, only the α- and β-forms have been extensively studied and characterized [9,32]. This study was therefore restricted to these two polymorphs.

Carbamazepine tablet formulations were prepared and their composition is given in Table 3. Formulations 1 and 2 contain only β-carbamazepine and α-carbamazepine, respectively. The individual tablet ingredients were weighed and mixed, first by the geometric dilution method and finally in the ball mill (without the ball). The required amount of powder mixture was weighed out and compressed in a hydraulic press (Fred S. Carver, Menomonee Falls, WI) to a pressure of 90 MPa and held for 1 min. The flat faced tablets were 11.2 mm in diameter and 2 mm thick. The XRD patterns of the intact tablets were obtained

Table 3
The composition of carbamazepine tablet formulations

Ingredient	Formulation (mg/tablet)			
	1	2	3	4
β-Carbamazepine	200.0		190.0	196.0
α-Carbamazepine		200.0	10.0	4.0
Microcrystalline cellulose	69.8	69.8	69.8	69.8
Sodium starch glycolate	11.0	11.0	11.0	11.0
Magnesium stearate	1.4	1.4	1.4	1.4
Silicon dioxide	1.4	1.4	1.4	1.4

using a specially fabricated holder [11]. The angular range of interest was 7–10°2θ and the scanning rate was 0.2°2$\theta \times \min^{-1}$. Comparison of the XRD patterns of β- and α-carbamazepine tablets revealed that the 10.10 Å line (peak at 8.75°2θ) was unique to α-carbamazepine (Fig. 5a, b). The XRD pattern of β-carbamazepine did not exhibit any peaks in the angular range in which this peak occurred. This line could therefore be used for the detection of α-carbamazepine in carbamazepine tablets.

Since the USP stipulates the use of the β-polymorph, it was of interest to determine the minimum detectable limit of α-carbamazepine in

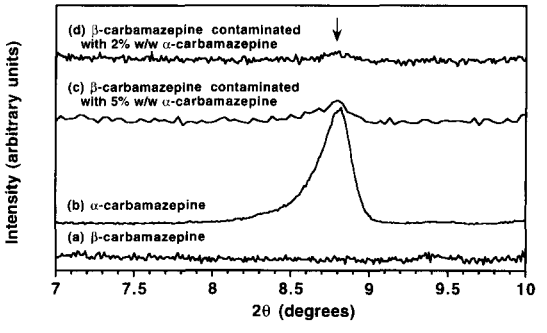

Fig. 5. (a) The XRD pattern of β-carbamazepine tablet. (b) The XRD pattern of α-carbamazepine tablet. The pronounced peak at 8.75°2θ (10.10 Å) is unique to this phase. (c) The XRD pattern of a tablet wherein 5% of the β-carbamazepine had been replaced with α-carbamazepine. (d) The XRD pattern of a tablet wherein 2% of the β-carbamazepine had been replaced with α-carbamazepine. The 10.10 Å line of α-carbamazepine is highlighted by the arrow. The full scale in (b) is different from that of (a), (c) and (d).

β-carbamazepine tablets. Tablets containing a mixture of α- and β-carbamazepine were prepared, wherein the α-carbamazepine content was $< 10\%$ w/w of the total carbamazepine content. At 5% w/w, α-carbamazepine was readily detected (Fig. 5c). It was possible to detect the α-polymorph down to a concentration of 2% w/w of the total carbamazepine content (1.4% w/w of the formulation). The composition of this formulation is given in Table 3 (formulation 3) and Fig. 5d is its XRD pattern. The signal to noise ratio in this case was 2.

3.7. Simultaneous identification of two active ingredients

Trimethoprim in combination with sulfamethoxazole is widely used for the treatment of a variety of infections. In these dosage forms, the ratio of trimethoprim to sulfamethoxazole is 1:5 (w/w). Tablets containing this drug combination permitted us to evaluate the utility of XRD method for the simultaneous identification of two active ingredients in a dosage form.

First of all, the XRD pattern of sulfamethoxazole was experimentally obtained (Table 4). The table also contains the sulfamethoxazole card pattern published by ICDD [33], which is data of the highest quality ('*' mark). A comparison of the XRD pattern of sulfamethoxazole obtained in our laboratory with the ICDD pattern reveals in general, a good agreement of the line positions. Two lines (d-spacings of 5.13 and 3.61 Å) are missing in the sulfamethoxazole XRD pattern obtained in our laboratory. This is an issue of resolution of closely spaced lines and was discussed in detail earlier. Similarly, the XRD pattern of trimethoprim also shows good agreement with the ICDD card pattern [34]. Incidentally, the ICDD has not assigned a quality mark to the trimethoprim pattern.

In physical mixtures of sulfamethoxazole and trimethoprim (5:1, w/w), some of the low intensity lines of trimethoprim are missing (Table 4). This is expected in light of the low weight fraction of trimethoprim (0.17) in these mixtures. Compared with the XRD pattern of sulfamethoxazole alone, only one line of very low intensity (d-spacing of 2.74 Å) was missing. In the commercial formulation (Bactrim™ tablet), the excipients were magnesium stearate, sodium starch glycolate, pregelatinized starch and coloring agents [18]. The weight fraction of the excipients is ~ 0.1. Their presence did not result in any new X-ray lines. All the high intensity lines (relative intensity $> 20\%$) observed in the powder pattern of the physical mixture were also observed in the tablet formulation (Table 4). However, some of the low intensity lines observed in the XRD pattern of the physical mixture were not observed in the pattern of tablets and vice versa.

3.8. Simultaneous identification of multiple active ingredients

The next object was to simultaneously identify three active ingredients in a dosage form. A commercially available tablet formulation was chosen (Excedrin®) which contains acetaminophen, aspirin and caffeine. The three active ingredients together constitute 83% w/w of the formulation. The XRD pattern contains numerous peaks in the angular range of 7–37°2θ (Fig. 6d). In an effort to identify the components in the dosage form, the XRD patterns of acetaminophen, aspirin and caffeine were obtained (Fig. 6a, b, c)). Acetaminophen could be readily identified by two unique lines with d-spacings of 6.48 and 4.92 Å (2θ values of 13.65 and 18.00°, respectively). At these 2θ values, the XRD patterns of aspirin and caffeine contain no peaks. Similarly, aspirin could be readily identified by two unique lines with d-spacings of 11.54 and 3.95 Å (2θ values of 7.65 and 22.45°, respectively). Caffeine has an intense line with a d-spacing of 7.55 Å (2θ value of 11.70°) and two intense lines with d-spacings of 3.39 and 3.31 Å (2θ values of 26.25 and 26.85°, respectively). Unfortunately, at these 2θ values, peaks due to aspirin and acetaminophen occur. Therefore, unambiguous identification was no longer possible. An added complication is that caffeine constitutes only 8.9% w/w of the formulation.

In an effort to identify caffeine, the XRD patterns of acetaminophen and aspirin were subtracted from the XRD pattern of the formulation.

Table 4
XRD data of sulfamethoxazole, trimethoprim, a mixture of sulfamethoxazole and trimethoprim powders and a marketed tablet formulation containing sulfamethoxazole and trimethoprim

ICDD card pattern of sulfamethoxazole (33)		Sulfamethoxazole powder		ICDD card pattern of trimethoprim (34)		Trimethoprim powder		Mixture of sulfamethoxazole and trimethoprim 5:1 w/w		Tablet containing sulfamethoxazole and trimethoprim[b]	
d-spacing (Å)	Relative intensity[a] (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity[a] (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)
				9.52	10	9.50	3			9.40	1
				8.42	75	8.46	3				
8.01	20	8.04	4					8.04	3	7.89	4
				7.44	57	7.49	28	7.49	2	7.31	30
7.16	60	7.16	19					7.16	11		
6.43	10	6.41	11					6.44	13		
				5.89	37	5.88	56	5.94	5		
				5.82	100	5.82	100	5.82	12	5.92	8
				5.49	11	5.54	6				
5.30	10	5.30	3					5.30	2	5.27	17
5.20	18	5.20	10					5.20	8		
5.13	25										
5.06	65	5.06	34	5.08	70	5.09	78	5.08	31	5.14	54
				4.92	20	4.99	14	4.95	1	4.94	7
4.88	8	4.88	3					4.88	2		
4.76	24	4.78	12	4.76	50	4.75	54	4.77	9	4.82	17
4.51	26	4.51	12	4.46	6	4.47	5	4.51	9	4.56	19
4.28	60	4.28	100					4.28	100	4.33	38
4.11	60	4.12	22					4.11	19	4.15	43
				4.03	8	4.04	27				
				3.98	46	3.99	57	3.99	46	4.00	20
3.95	25	3.96	14					3.95	13	3.91	2
3.75	45	3.74	30	3.74	16	3.74	73	3.75	19		
3.71	100	3.72	58					3.72	38	3.75	100
3.61	10										
3.59	15	3.60	9					3.60	6	3.62	10
				3.53	10	3.53	21			3.49	5
				3.49	6	3.47	28			3.45	6
				3.43	74	3.43	69	3.42	4	3.42	5
3.24	45	3.25	28					3.25	21	3.27	35
3.09	20	3.09	13					3.10	11	3.07	4
				2.90	10	2.91	15	2.90	3	2.90	2
2.77	20	2.77	14					2.77	10	2.79	17
2.74	10	2.75	5							2.75	4
				2.67	10	2.68	6	2.68	2	2.66	4

[a]Only lines with relative intensities >5% are considered here.
[b]Bactrim™.

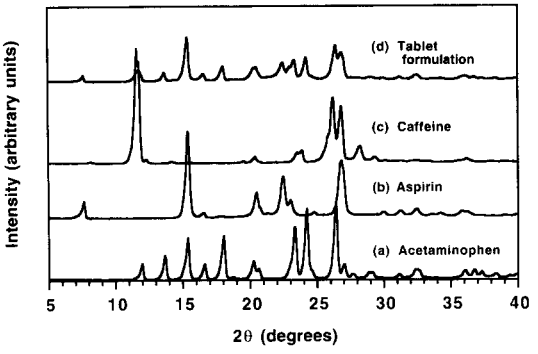

Fig. 6. The XRD pattern of acetaminophen powder. (b) The XRD pattern of aspirin powder. (c) The XRD pattern of caffeine powder. (d) The XRD pattern of a powdered tablet formulation containing acetaminophen, aspirin and caffeine (Excedrin®).

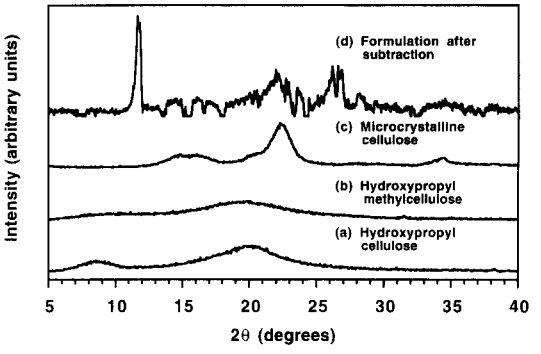

Fig. 8. (a) The XRD pattern of hydroxypropyl cellulose powder. (b) The XRD pattern of hydroxypropyl methylcellulose powder. (c) The XRD pattern of microcrystalline cellulose powder. (d) The residual XRD pattern after proportional subtraction of acetaminophen and aspirin XRD patterns from that of the powdered tablet formulation.

The pattern subtraction technique was described earlier. Acetaminophen and aspirin each constitute 37% w/w of the formulation. When the subtracted profile (Fig. 7a) is compared with that of caffeine (Fig. 7b), the high intensity peaks of caffeine at 11.70, 26.25 and 26.85°2θ were readily discernible. However, an amorphous halo was observed over the angular range of 18–23°2θ. The formulation contains numerous excipients which include microcrystalline cellulose, hydroxypropyl methylcellulose and hydroxypropyl cellulose [18]. The XRD pattern of microcrystalline cellulose exhibited a broad halo over the angular range of 18–25°2θ (Fig. 8c). While amorphous halos were also observed in the XRD patterns of hydroxypropyl methylcellulose (Fig. 8b) and hydroxypropyl cellulose (Fig. 8a), their angular range did not match that of the formulation (Fig. 8d). Therefore, microcrystalline cellulose is likely to be the major contributor to the observed halo. Thus XRD not only permitted simultaneous identification of all the active ingredients in the dosage form but it also provided information about the excipients in the formulation. In order for the pattern subtraction to be meaningful, the samples were milled (Spex Mixer/Mill, Metuchen, NJ) so that the final particle size was < 150 μm. The milling did not cause any phase transformations.

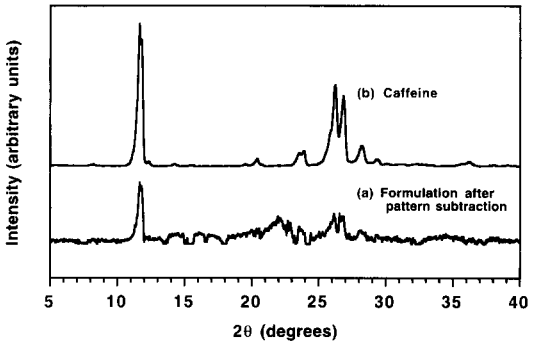

Fig. 7. (a) The residual XRD pattern after proportional subtraction of acetaminophen and aspirin XRD patterns from that of the powdered tablet formulation. (b) The XRD pattern of caffeine powder.

4. Discussion

Since the pioneering work of Shell [8], the use of XRD for the identification of the active ingredient in dosage forms has been attempted [21,35–44]. However, the indexing of the XRD pattern was carried out manually and its reliability is therefore questionable. More importantly, the authors did not perform a careful, line-by-line comparison of the experimentally obtained XRD pattern with the patterns published by the ICDD. Therefore, one cannot evaluate the quality of the data obtained.

The technique of XRD offers numerous advantages. It is not only simple and direct but it also

permits unambiguous identification of the drug. In addition, the technique provides information about the solid-state of the drug (polymorphic form, state of solvation and degree of crystallinity). The dosage form is analyzed after minimal or no sample pretreatment and there is no need to separate the active ingredient from the excipients in the formulation. The identification of the active ingredient can be accomplished without any knowledge of the other ingredients in the formulation. As demonstrated, simultaneous identification of more than one active ingredient in the formulation was possible.

There are several potential applications of the technique. Alterations in solid-state induced during pharmaceutical processing, such as polymorphic transitions or changes in the degree of crystallinity can be detected and even quantified. The technique can nondestructively distinguish between drug containing and placebo formulations which is of potential utility in double blind clinical studies [45].

There are some limitations of the technique. The foremost requirement is that the active ingredient be crystalline in the formulation. Moreover, it should constitute a significant weight fraction of the formulation. Our preliminary studies suggest that the crystalline drug should constitute at least 5% w/w of the formulation.

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