Simultaneous Quantification of an Enantiomer and the Racemic Compound of Ibuprofen by X-ray Powder Diffractometry

Neelima V. Phadnis[1] and Raj Suryanarayanan[1,2]

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Purpose. An X-ray powder diffractometric method was developed for the simultaneous quantification of the relative amounts of the racemic compound (±) of ibuprofen (I) and S(+)-ibuprofen (II), when they occur as a mixture.

Method. The X-ray powder diffraction patterns of I and II show pronounced differences. This formed the basis for the determination of the relative amounts of I and II when they occur as a mixture. X-ray lines with d-spacings of 14.41 and 4.37 Å were unique to I and II, respectively. Mixtures containing different proportions of I and II were prepared which also contained lithium fluoride (III) as an internal standard.

Results. A linear relationship was obtained when the intensity ratio (intensity of the 4.37 Å line of II/intensity of the 2.01 Å line of III) was plotted as a function of the weight fraction of II in the mixture. Similar results were obtained in the case of I. Using these standard curves, the weight fractions of I and II in "unknown" mixtures were determined. The experimentally determined analyte concentration ranged between 98 and 104% of the true value. The relative error in the analyses of individual samples was <10%. The minimum detectable weight fraction of I in II and II in I were 0.032 (3.2% w/w) and 0.034 (3.4% w/w), respectively. The minimum quantifiable weight fractions were 0.136 for I and 0.112 for II. Since the X-ray diffraction patterns of S(+)-ibuprofen and R(−)-ibuprofen are identical, the conclusions drawn regarding mixtures of I and II will also hold true in the quantitative analyses of mixtures of I and R(−)-ibuprofen.

KEY WORDS: S(+)-ibuprofen; R(−)-ibuprofen; (±)-ibuprofen; X-ray powder diffractometry.

INTRODUCTION

Stereoisomerism is observed in compounds which contain chiral centers. Enantiomers are stereoisomers which are nonsuperimposable mirror images of each other. An equimolar mixture of the two enantiomers is called a racemate. A racemate can be classified into three types, (i) racemic compound, (ii) racemic mixture or conglomerate, and (iii) racemic solid solution or pseudoracemate (1). In a racemic compound, equimolar amounts of the two enantiomers are present in each racemic crystal. The racemic conglomerate on the other hand is a physical mixture of crystals of the two pure enantiomers. When the two enantiomers form a continuous solid solution, it is termed as a pseudoracemate.

The storage temperature can influence the type of racemate present (1,2). While tartaric acid exists as a racemic mixture below 26°C, it is a racemic compound above this temperature (2). The nature of a crystalline racemate may not only have profound implications on its physicochemical properties but also influence its *in vivo* performance. For example, individual enantiomers of ibuprofen have a higher water solubility and dissolve faster than the racemic compound (3). Therefore the absorption characteristics of a racemic mixture could be different from that of the racemic compound.

Though the physicochemical properties of a racemic compound and a racemic mixture are different, these differences are lost following dissolution or fusion (2). Chiral chromatographic techniques, despite their high sensitivity, are incapable of distinguishing between the racemate types. Infrared (IR) spectroscopy, differential scanning calorimetry (DSC), solid-state nuclear magnetic resonance (NMR) spectroscopy and X-ray powder diffractometry are potentially useful to distinguish between racemic mixtures and racemic compounds (4).

Enantiomers have identical XRD patterns since their crystal structures are mirror images of each other. The unit cell parameters of an enantiomer and the corresponding racemic compound are different, resulting in different XRD patterns. This dissimilarity can be exploited for both phase identification and quantification.

Ibuprofen, a widely used analgesic and antiinflammatory agent, was selected as the model compound. Under ambient storage conditions, ibuprofen can exist as the S(+)-enantiomer, R(−)-enantiomer and the racemic compound (hereafter referred to as the (±)-form). The currently marketed formulations contain (±)-ibuprofen. Formulations containing S(+)-ibuprofen have recently been introduced in the European market. A switch from the racemic compound to the S(+)-enantiomer is also under consideration in the United States (5). This enantiomer is manufactured in bulk by enzymatic synthesis (6). If this process yields the R(−)-enantiomer as an impurity, under certain circumstances, it is expected to form a racemic compound in the solid-state (4). Therefore, the goal of this project was to demonstrate the utility of X-ray powder diffractometry to determine the relative amounts of S(+)- and (±)-ibuprofen when they occur as mixtures.

MATERIALS AND METHODS

Materials

(±)-Ibuprofen and S(+)-ibuprofen were gifts from Ethyl Corporation (Baton Rouge, LA) and lithium fluoride was obtained from Aldrich Chemical Co. (Milwaukee, WI). All these materials were stored at room temperature (~25°C) under 0% relative humidity (RH).

Preparation of Mixtures

The S(+)- and (±)-ibuprofen were each milled in a ball mill (Spex Mixer/Mill, Spex Industries, Metuchen, NJ) for 10 minutes using a sample holder and ball made of agate. The milled samples were stored for at least 48 hours at ~0% RH (anhydrous calcium sulfate) before use.

Different proportions of S(+)- and (±)-ibuprofen were mixed with lithium fluoride (20% w/w) by the geometric dilution technique. At each composition, three sample mixtures were prepared and immediately subjected to XRD.

[1] College of Pharmacy, 308 Harvard Street S.E., University of Minnesota, Minneapolis, Minnesota 55455.

[2] To whom correspondence should be addressed. (e-mail: surya001@maroon.tc.umn.edu)

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Powder X-ray Diffractometry

Samples were exposed to CuKα radiation (35 kV × 50 mA) in a wide-angle powder X-ray diffractometer (Model D Max B, Rigaku). The Bragg-Brentano focusing geometry was used, with a 1° divergence slit, a 1° scatter slit, a 0.3° receiving slit, and a scintillation counter as the detector. The diffractometer was operated in the step-scan mode in increments of 0.01°2θ (2 seconds/step) over three angular ranges. (i) From 5.00 to 6.70°2θ to determine the intensity of the 14.41 Å line unique to (±)-ibuprofen. The peak intensity was usually determined by integrating between 5.70 and 6.45°2θ. Background counts were usually obtained over two angular ranges—5.10 to 5.18°2θ and 6.50 to 6.58°2θ. (ii) From 20.30 to 21.70°2θ to determine the intensity of the 4.37 Å line unique to S(+)-ibuprofen. The peak intensity was usually determined by integrating between 20.70 and 21.60°2θ. In this case, background counts were usually obtained over only one angular range—20.45 to 20.70°2θ. (iii) From 43.30 to 45.70°2θ to determine the intensity of the 2.01 Å line unique to lithium fluoride. The peak intensity was usually determined by integrating between 44.17 and 45.43°2θ. Background counts were usually obtained over two angular ranges—43.63 to 43.70°2θ and 45.60 to 45.67°2θ.

The area under the curve (AUC) of each peak was determined after appropriate background subtraction (7). In the immediate vicinity of the 14.41 Å line (peak at 6.10°2θ) of (±)-ibuprofen and the 2.01 Å (peak at 45.10°2θ) line of lithium fluoride, there are no interfering peaks (Fig. 1). This permitted the determination of background counts on both sides of the peak. As is evident from Fig. 1, such a background correction was not possible in the case of the 4.37 Å (peak at 21.30°2θ) line of S(+)-ibuprofen. In this case, background subtraction was performed using background counts from only one side of the peak.

Sample Preparation for Powder X-ray Diffractometry

The sample holder was made of aluminum and consisted of a rectangular central cavity, 15.5 mm × 19.5 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 (8,9).

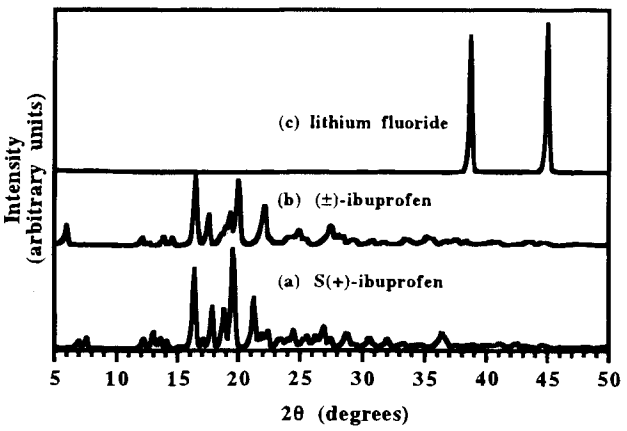

Fig. 1. The powder X-ray diffraction patterns of (a) S(+)-ibuprofen, (b) (±)-ibuprofen, and (c) lithium fluoride.

RESULTS AND DISCUSSION

The theoretical basis of quantitative powder X-ray diffractometry was developed by Alexander and Klug (7,10). Suppose a system consists of an unknown component J and an internal standard S. The line i of component J (i.e. analyte), I_{iJ}, and line k of component S, I_{kS}, are chosen for quantitative analysis. The intensity ratio of the two lines is given by Eq. 1.

$$\frac{I_{iJ}}{I_{kS}} = Kx_J \quad (1)$$

where K is a constant and x_J is the weight fraction of the unknown component. When the internal standard is added in a constant proportion, the intensity ratio, I_{iJ}/I_{kS}, is a linear function of the weight fraction of component J, x_J.

Guidelines for the selection of an internal standard for XRD are outlined by Shell (11) and are reviewed in detail by Suryanarayanan (12). Lithium fluoride was used as the internal standard in the analysis of ibuprofen mixtures. It belongs to the cubic crystal system and has few, but intense diffraction peaks (Fig. 1).

Ibuprofen Mixtures

The powder X-ray diffraction patterns of (±)-ibuprofen and S(+)-ibuprofen are shown in Fig. 1. The powder pattern of (±)-ibuprofen was identical to that reported in the powder diffraction files (PDF) of the International Centre for Diffraction Data (13). The PDF database does not contain the powder patterns of S(+)- and R(−)-ibuprofen. However, the powder pattern of S(+)-ibuprofen matched that of S(+)-ibuprofen reported in the literature (3).

When the powder X-ray patterns of (±)-ibuprofen and S(+)-ibuprofen were compared (Fig. 1), it was evident that in the angular range where the 14.41 Å line of (±)-ibuprofen occurred (peak at 6.1°2θ), there were no peaks in the powder pattern of S(+)-ibuprofen. The 14.41 Å line is due to diffraction by the plane with Miller indices (100) (13). Similarly, in the angular range where the 4.37 Å line of S(+)-ibuprofen occurred (peak at 21.30°2θ), there were no peaks in the powder pattern of (±)-ibuprofen. These conclusions are also valid for R(−)-ibuprofen, since the XRD patterns of S(+)-ibuprofen and R(−)-ibuprofen are identical. Therefore, from now on, it is implicitly assumed that the conclusions drawn in the case of mixtures of (±)-ibuprofen and S(+)-ibuprofen are also applicable to mixtures containing (±)-ibuprofen and R(−)-ibuprofen.

In addition to XRD, (±)-ibuprofen and S(+)-ibuprofen were also characterized by Karl Fischer titrimetry (model CA-05 Moisture Meter, Mitsubishi) and by differential scanning calorimetry (model 910, TA Instruments). Based on Karl Fischer titrimetry, the water content of both S(+)-ibuprofen and (±)-ibuprofen was determined to be <0.1% w/w. The enthalpy of fusion of S(+)-ibuprofen was determined to be 18.8 ± 0.3 $kJ \cdot mol^{-1}$ (mean ± SD; n = 3). This was in reasonably good agreement with the value of 19.9 ± 0.8 $kJ \cdot mol^{-1}$ reported in literature (3). The experimentally determined enthalpy of fusion of (±)-ibuprofen, 25.8 ± 0.2 $kJ \cdot mol^{-1}$, was in good agreement with the reported value of 26.9 ± 1.0 $kJ \cdot mol^{-1}$ (3). Incidentally,

neither the enantiomers nor the racemic compound exhibit polymorphism. There are also no reports of pseudoracemate formation (3).

The intensity ratio (intensity of the 4.37 Å line of S(+)-ibuprofen/intensity of the 2.01 Å line of lithium fluoride) was plotted as a function of the weight fraction of S(+)-ibuprofen in the mixture. A linear relationship was observed (Fig. 2). A similar linear relationship was observed when the intensity ratio (intensity of the 14.41 Å line of (±)-ibuprofen/intensity of the 2.01 Å line of lithium fluoride) was plotted as a function of the weight fraction of (±)-ibuprofen in the mixture (data not shown). The equation of this line was:

$$\textit{intensity ratio} = -0.0203 + 0.6714 * (\textit{weight fraction}) \quad (r^2 = 0.99) \tag{2}$$

where "weight fraction" is the weight fraction of (±)-ibuprofen in the mixture.

Quality-control mixtures of known composition were prepared. In these mixtures, the intensities of the 14.41, 4.37 and 2.01 Å lines of (±)-ibuprofen, S(+)-ibuprofen and lithium fluoride respectively were determined. From the experimentally obtained intensity ratios, the (±)-ibuprofen and S(+)-ibuprofen content were determined using Eq. (2) and Fig. 2, respectively (Table I). The experimentally determined analyte concentration ranged between 98 and 104% of the true value. The relative error in the analyses of individual samples was <10% (Fig. 3).

Limit of Detection and Quantification

The minimum distinguishable analytical signal, S_m, is expressed as:

$$S_m = \bar{S}_{bl} + ks_{bl} \tag{3}$$

where $\bar{S}_{bl}$ = mean blank signal, k = a multiple, for which a reasonable value is 3, and s_{bl} = standard deviation of the blank signal (14,15).

The blank signal can be obtained from the background counts of samples in which the analyte weight fraction is 0. In order to determine the limit of detection of S(+)-ibuprofen in (±)-ibuprofen, mixtures of (±)-ibuprofen and lithium fluoride were prepared. Lithium fluoride constituted 20% w/w of the mixture. The background counts were obtained by integrating between 20.70 and 21.60°2θ. As mentioned earlier, this was the approximate angular range over which the intensity of the 4.37 Å line of S(+)-ibuprofen was obtained. The background subtracted intensity of the 2.01 Å line of lithium fluoride was obtained according to the procedure described in the Experimental section. The blank signal is based on these two determinations and is expressed as:

Table I. Accuracy in the Analyses of Ibuprofen Mixtures (n = 3)

Component (Ibuprofen)	Actual weight fraction in mixture	Calculated mean weight fraction	% of true weight fraction	Coefficient of variation (%)
S(+)-enantiomer	0.150	0.153	102	5.3
	0.250	0.253	101	1.8
	0.500	0.510	102	6.7
	0.750	0.756	101	6.1
	0.850	0.867	102	3.5
	0.900	0.879	98	2.8
Racemic compound	0.100	0.104	104	7.8
	0.150	0.153	102	7.3
	0.250	0.250	100	2.8
	0.500	0.491	98	5.6
	0.750	0.749	100	4.4
	0.850	0.857	101	7.5

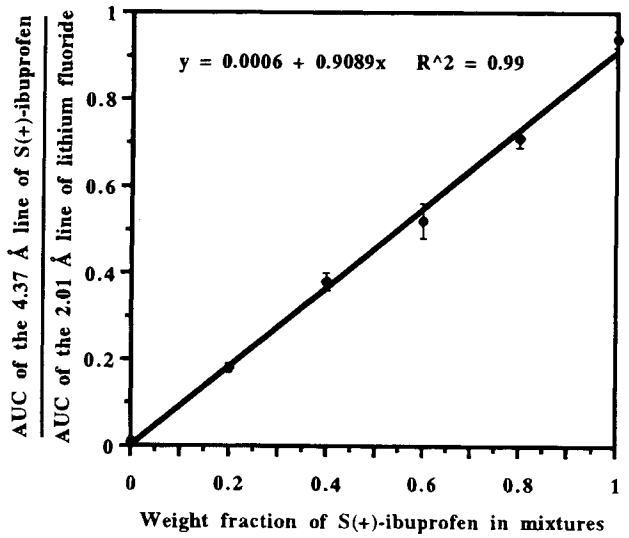

Fig. 2. Plot of the intensity ratio (intensity of the 4.37 Å line of S(+)-ibuprofen/intensity of the 2.01 Å line of lithium fluoride) as a function of the weight fraction of S(+)-ibuprofen in mixtures containing S(+)-ibuprofen and (±)-ibuprofen. All the mixtures contained lithium fluoride (20% w/w) as the internal standard. Since its composition was constant, it was not taken into consideration in the weight fraction calculations.

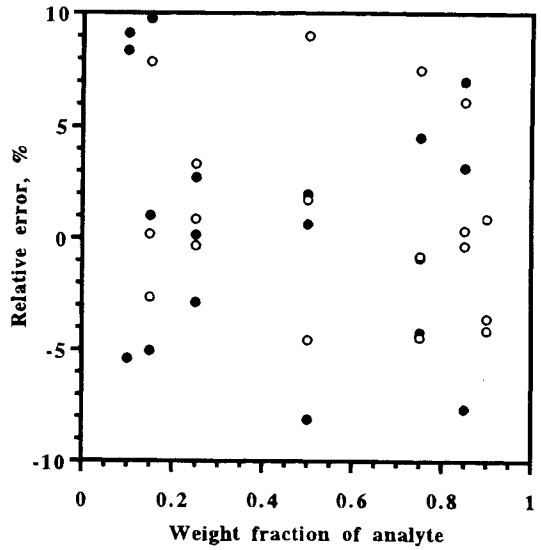

Fig. 3. The relative error in the determination of ibuprofen content in the mixtures; (○) S(+)-ibuprofen; (●) (±)-ibuprofen.

$$S_{bl} = \frac{\text{intensity over the angular range of 20.70 to 21.60° 2}\theta}{\text{intensity of the 2.01 Å line of lithium fluoride}} \quad (4)$$

Several determinations of S_{bl} (n = 9) were performed, from which $\bar{S}_{bl}$ and s_{bl} were calculated.

The relationship between the measured signal, S, and the analyte concentration, C, can be described by the equation:

$$S = mC + S_{bl} \quad (5)$$

where, m = slope, and S_{bl} = instrumental signal for blank. In our system, S is expressed as an intensity ratio. The equation describing the relationship between the intensity ratio and the analyte concentration (S(+)-ibuprofen) is given in Fig. 2.

At the minimum concentration at which a measurable signal is obtained, Eq. 5 can be modified to:

$$S_m = mC_m + \bar{S}_{bl} \quad (6)$$

where C_m is the limit of detection.

By substituting the values of S_m, $\bar{S}_{bl}$ and m into Eq. 6, the minimum detectable weight fraction of S(+)-ibuprofen in (±)-ibuprofen was calculated to be 0.034 (3.4% w/w).

To determine the limit of detection of (±)-ibuprofen in S(+)-ibuprofen, mixtures of S(+)-ibuprofen and lithium fluoride were prepared. In this case, the background counts were obtained by integrating between 5.70 and 6.45°2θ. The rest of the procedure was the same as described earlier. The minimum detectable weight fraction of (±)-ibuprofen in S(+)-ibuprofen was 0.032 (3.2% w/w).

The limit of quantification can be defined as ten times the standard deviation of the blank signal ($10 \times s_{bl}$) (15). The minimum quantifiable weight fractions of (±)-ibuprofen and S(+)-ibuprofen were calculated to be 0.136 (13.6% w/w) and 0.112 (11.2% w/w), respectively.

The possible sources of error in quantitative powder X-ray diffractometry were considered (12). As mentioned in the Experimental section, it was necessary to grind (±)-ibuprofen and S(+)-ibuprofen in a ball mill. Microscopic examination of the milled samples revealed that the particles were <50 μm (longest dimension) in size. The use of such fine particles should aid in minimizing preferred orientation of the particles. However, milling can cause alterations in the solid-state of the materials. The XRD patterns of the samples before and after milling were identical. Therefore milling did not cause any detectable phase transitions in the sample.

Milling can also result in more subtle alterations in solid-state. For example, milling is known to cause a decrease in the degree of crystallinity (i.e. decrease in lattice disorder). The integrated X-ray peak intensity has been reported to be inversely dependent on the degree of lattice disorder (16). Milling did not cause any detectable alterations in the degree of crystallinity. This conclusion was based on three observations. (i) The XRD patterns of the milled samples did not contain any amorphous halos. (ii) The background counts in the milled and unmilled samples were virtually identical. (iii) The heat of fusion of milled and unmilled samples were not significantly different and matched those reported in the literature (3).

Because of the quantitative nature of the work, we wanted to be absolutely certain that the milling process did not affect the intensity measurements. If milling causes activation of a solid, recrystallization of the activated regions can occur during storage. This may cause changes in the integrated intensities of the X-ray lines as a function of storage time (16). The (±)-ibuprofen and S(+)-ibuprofen were each milled for 10 minutes and stored at ambient temperature under ~0% RH. The integrated intensity of the characteristic lines of (±)-ibuprofen and S(+)-ibuprofen were monitored as a function of time [Fig. (4)]. The AUC of the 14.41 Å line of (±)-ibuprofen in the freshly milled sample was not significantly different from that obtained after storage for 6, 15 and 24 hours (ANOVA; $\alpha = 0.05$). A similar observation was made with the 4.37 Å line of S(+)-ibuprofen. Therefore, we concluded that grinding did not cause even subtle alterations in the solid-state of the materials.

Several analytical techniques are available for the quantitative analyses of chiral systems (17). The techniques can be broadly classified as those that require dissolution of the material under study, e.g. chiral chromatography, and those that examine it in the solid-state, e.g. XRD. Chiral chromatography is known to be a sensitive technique for the detection of low levels of contamination of one enantiomer by its opposite enantiomer. For example, 0.05% w/w of S-enantiomer of leucine was detected in R-leucine by chiral gas liquid chromatography (18). Chiral chromatographic techniques, despite their high sensitivity, are incapable of distinguishing between the racemate types. Since XRD can distinguish between racemic mixtures and racemic compounds, it does not suffer from any such ambiguity. In fact, since 90 to 95% of crystalline racemates occur as racemic compounds (2), XRD can serve as a *reference analytical method* in these instances. However, it must be pointed out that XRD cannot distinguish between the S(+) and R(−)-ibuprofen. The second limitation of XRD is that it is much less sensitive than chiral chromatography. However this problem is not unique to XRD and is often encountered when analyses is performed in the solid-state.

CONCLUSIONS

A simple XRD method for the quantitative analyses of mixtures of S(+)-ibuprofen and (±)-ibuprofen has been devel-

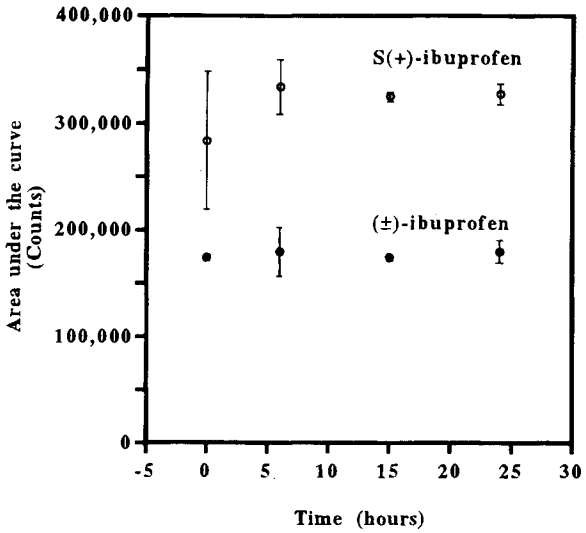

Fig. 4. The area under the curve as a function of storage time after milling; (○) 4.37 Å line of S(+)-ibuprofen; (●) 14.41 Å line of (±)-ibuprofen. Error bars represent standard deviations (n = 3).

oped. Unlike conventional analytical techniques, this method enables quantification in the solid-state. Therefore, it is an excellent complement to highly sensitive solution based techniques such as chiral chromatography. The sample preparation was extremely simple and only involved addition of an internal standard. The XRD pattern of R(−)-ibuprofen is identical to that of S(+)-ibuprofen. Therefore, the conclusions from the analysis of S(+)-ibuprofen mixtures with (±)-ibuprofen will also hold true for mixtures of R(−)-ibuprofen with (±)-ibuprofen.

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