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Quantitation of cefepime·2HCl dihydrate in cefepime·2HCl monohydrate by diffuse reflectance IR and powder X-ray diffraction techniques

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Abstract

The identification, characterization and quantitation of crystal forms is becoming increasingly important within the pharmaceutical industry. Multi-disciplinary, physical analytical techniques are necessary for this task. In this work, diffuse reflectance mid-infrared (IR) and powder X-ray diffraction (XRD) analyses were used to identify two different hydrated forms of cefepime·2HCl, a cephalosporin. Characterization of the mono- and dihydrate forms led to separate IR and XRD quantitative assays for the determination of dihydrate content in cefepime·2HCl monohydrate bulk material. For the IR assay, a working range of 1.0–8% (w/w) was established with a minimum quantifiable level (MQL) of 1.0% (w/w) and a limit of detection (LD) of 0.3% (w/w) dihydrate in monohydrate material. The XRD assay displayed a working range of 2.5–15% (w/w) with an MQL of 2.5% (w/w) and an LD of 0.75% (w/w). Cross validation was performed between the two techniques, with a good correlation displayed for each assay as compared with the known concentrations and as compared with each other. In addition, a full evaluation of potential assay errors was made.

Keywords: Quantitative analysis of hydrates; Cefepime·2HCl monohydrate; Cefepime·2HCl dihydrate; Infrared spectroscopy; Powder X-ray diffraction

1. Introduction

Polymorphism is a well understood and key element in the drug development and manufacturing process for pharmaceutical compounds of interest [1,2]. Various crystal forms of a drug substance (including anhydrates, hydrates and solvates) may display significantly different physical properties, such as melting point, solubility, density, morphology, stability and bioavailability. For this reason, it is critical to identify, characterize and, whenever possible, quantitate the presence of various solid-state forms of a pharmaceutical compound.

Typically, the most stable and efficacious form is selected for the formulation process. However, sometimes this form is not the easiest to manu-

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facture. It is therefore necessary to characterize bulk material for its crystal form content. Since different solid-state forms may display variations in the bulk stability upon exposure to stress conditions, the crystal form content must be monitored at this stage of the development/manufacture process. In addition, it is widely recognized that formulation of the drug may transform the substance into a different form. Various factors such as thermal and mechanical energy from tabletting processes [3,4] or mode of blending (dry slugging versus wet granulation) may induce crystallographic transformation [5].

A multitude of physical analytical techniques have been used to characterize crystal forms. Typically, powder X-ray diffraction (XRD) [6] and microscopy techniques [7] are initially employed, with further studies utilizing solubility measurements [8] and thermal analysis techniques such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) [9]. More recently, spectroscopic techniques such as infrared (IR) [10–12], Raman [13,14] and solid-state nuclear magnetic resonance (NMR) [10,15,16] have been used. It must be recognized that no one technique may be used to characterize fully a solid-state system. Various physical analytical techniques have advantages and disadvantages when compared with each other. For this reason, a multi-disciplinary approach to the identification, characterization and quantitation of pharmaceutical forms must be taken.

In this paper, the issue of quantitation of one hydrate within another at the bulk level is examined. Studies on the cephalosporin cefepime·2HCl have led to the discovery of at least two crystalline hydrated forms, a monohydrate and dihydrate. The structure of cefepime • 2HCl monohydrate is shown in Fig. 1. Utilizing a multidisciplinary approach, the two solid-state forms of the drug were investigated by XRD, DSC, TGA, mid-IR, NMR, optical microscopy and solubility studies. Thermal analysis studies verified the existence of the mono- and dihydrate forms, further collaborated by hot-stage microscopy and water content determination by Karl Fischer titration. Distinct XRD, diffuse reflectance IR and ^{13}C solid-state NMR data were collected for the two forms.

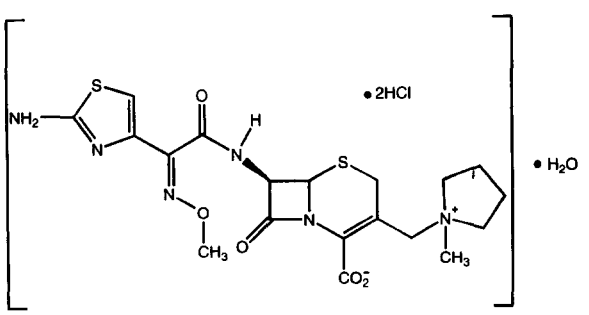


Fig. 1. Structure of cefepime·2HCl monohydrate.

In support of the new drug application (NDA) for the monohydrate form, a quantitative assay was required for the determination of the content of cefepime·2HCl dihydrate form in batches of monohydrate material. Requirements for this assay included (a) assurance that crystallographic transformation did not occur during sample preparation or analysis, (b) a detection limit of approximately 5% (w/w) dihydrate material in monohydrate batches, (c) ease of sample preparation and subsequent data acquisition and analysis and (d) the ability to perform the assay in a quality control (QC) environment. Based upon these requirements and results from the multidisciplinary characterization studies, quantitative assays were developed utilizing mid-IR and XRD techniques.

One of the critical factors in developing any solid-state form assay is the generation of authentic calibration and validation samples which simulate actual material that will be assayed in the future. One of the greatest difficulties in assay development is not the ability to procure genuine crystallographic material, but the ability to utilize homogeneously mixed samples during the generation of calibration and validation data. To this end, a slurry technique was used in our laboratories to produce authentic calibration/validation samples that are homogeneous. Utilization of this approach in the quantitation of the dihydrate content in cefepime·2HCl monohydrate material fully satisfied the aforementioned assay requirements.

2. Experimental

2.1. Sample preparation

2.1.1. Materials

Authentic cefepime·2HCl mono- and dihydrate were obtained from the Chemical Process Development Laboratories of the Bristol-Myers Squibb Pharmaceutical Research Institute. The batches of material used for method development displayed a chemical purity in excess of 99% as determined by high-performance liquid chromatography (HPLC).

2.1.2. Particle size distribution

It is widely recognized that diffuse reflectance IR [17] and XRD measurements [18] are particle size dependent techniques. For this reason, the particle size distribution of mono- and dihydrate materials was restricted to the range 125–590 μm. The particle size distribution range was maintained by passing the separate materials through five vibrating sieves (30, 40, 60, 80 and 120 mesh). Only the material retained on these five sieves was used for method development.

2.1.3. Slurry preparation

Since one of the prerequisites for assay development was a detection limit $\leqslant 5\%$ (w/w), the calibration samples were limited to a working range of 1–15% (w/w). As previously mentioned, the generation of homogeneously mixed samples is critical to any form of solid-state analysis. To this end, sample preparation involved the use of an acetone slurry to mix the two materials homogeneously. Each respective calibration, validation or reproducibility sample was prepared by weighing out a specific amount of the dihydrate material into a 10 ml glass vial and adding the appropriate amount of monohydrate material to give a final weight of approximately 300 mg of sample. This amount of material was sufficient for concurrent IR and XRD analysis. A slurry of each sample was then prepared by adding 8 ml of acetone to the mixture contained within the vial. The vial was capped and agitated for 5 min to form a slurry. The slurry was then filtered using a 0.22 μm Millipore Fluoropore filter, with the vacuum being pulled for an additional 5 min to dry the sample on the filter. The sample and filter were then placed in the original glass vial and placed in a vacuum desiccator. The desiccator chamber containing the samples was evacuated for at least 1 h to remove any residual acetone from the samples. The samples were then removed from the filter and blended with a spatula to break up the filter cake into a powder. Subsequent gas chromatographic analysis of the samples confirmed that minimal residual acetone remained with the samples.

2.2. Instrumentation

2.2.1. Diffuse reflectance mid-IR spectroscopy

The diffuse reflectance (DR) infrared Fourier transform spectra were acquired on a Nicolet Model 740 Fourier transform infrared (FTIR) spectrophotometer with the use of a Spectra-Tech diffuse reflectance accessory unit. A water-cooled Globar source was used in conjunction with a Ge/KBr beamsplitter and a liquid nitrogen-cooled, narrow-band mercury cadmium telluride detector (MCT-a). Before the acquisition of experimental data, the IR and DR unit were aligned in accordance with the manufacturers' instructions. Each spectrum represents 64 co-added scans obtained at a spectral resolution of 4 cm^{-1}. In order to minimize spectral absorptions due to atmospheric gases, a nitrogen purge was maintained in the optical bench. A 5 min equilibration time was used between the introduction of a new sample into the spectrophotometer and actual data acquisition. The macro sample cup (13 mm diameter) was used to hold the neat samples (no alkali metal halide diluent used). Each cup was filled with the use of a spatula and then leveled by lightly pressing a glass slide downward upon the sample until the sample height matched the cup height. A sample cup filled with dried KBr was used as the background data set. Digital ratioing of the sample data set against the background set and subsequent processing produced a frequency domain spectrum represented in reflectance units [$\log(1/R)$].

2.2.2. X-ray powder diffraction

XRD measurements were obtained on a Philips Model APD 3720 powder diffraction system, equipped with a vertical goniometer in $\theta/2\theta$ geometry. The generator (Philips Model XRG 3100) was operated at 45 kV and 40 mA, using copper Kα radiation. The intensity of the X-rays was regularly monitored with an external silicon standard. Philips APD software version 4.00 was used for all data collection and data analysis. To minimize the effect of compaction on the sample preparation, all samples were weighed before being packed into the sample holders. Between 0.242 and 0.248 g were used for all samples, and the samples were backfilled into the sample holders to minimize preferential orientation. The reflection at approximately 13.7° 2θ was used for quantitation of the cefepime dihydrate form. A step scan was recorded for all samples from 12 to 15° 2θ, with a step size of 0.02° 2θ and a count time of 3 s. The peak heights of the dihydrate peak were determined by the profile fitting program of the Philips software. A straight-line quantitative model was employed for the calibration (see below). Qualitative scans were collected at a scan rate of 0.04° 2θ and a time of 1 s per step in the range 2–32° 2θ.

2.2.3. Scanning electron microscopy (SEM)

SEM images were obtained on an Amray 1820T system, using an acceleration potential of 20 kV. The samples were sputter-coated with Au/Pd to eliminate charging effects.

2.2.4. True powder density

The true powder density of the materials was detemined using a Quantachrome Multipycnometer. Approximately 1.5 g of material was weighed out and placed in a sample cell. Helium gas was used to pressurize the sample chamber and pressure readings were used to obtain the true volume (ml) of the sample. The true density (g ml^{-1}) was calculated by dividing the mass of the sample by the volume measured.

2.3. Assay error evaluation

In order to ascertain errors associated with the DR IR or XRD assay, samples were prepared to investigate the following parameters.

2.3.1. Instrument reproducibility

This source of error was investigated by placing a single mixture of sample in the IR (5.9%, w/w) and XRD (4.7%, w/w) instruments and acquiring six data sets without removing the sample from the sample cup/holder or instrument.

2.3.2. Day-to-day reproducibility

Variability in instrument response was monitored for 4 days on the IR and 6 days on the XRD instrument. A single mixture of sample (IR 5.9%, w/w; XRD, 5.0%, w/w) was placed in each instrument and a single data set acquired each day.

2.3.3. Sample positioning

The effect of the position of the sample cup in the DR mid-IR accessory unit or the holder in the XRD autosampler was determined. A single packing of a sample (4.7%, w/w) was randomly placed in the DRIFTS and XRD instrument 10 different times and data sets were acquired for each.

2.3.4. Sample packing

Variation due to crystal orientation was investigated by repacking the same mixture into a sample cup/holder 10 different times and 10 different data sets were acquired (IR, 6.0%, w/w; XRD, 7.6%, w/w).

2.3.5. Sample mixing

Variation of sample mixing was determined by preparing a single mixture of sample (5.3%, w/w) by the slurry technique and analyzing 10 subsamples of this concentration by DR IR and XRD.

2.3.6. Method error

The relative standard deviation (RSD) for the method was calculated by assaying one sample 10 times by DR IR and XRD and utilizing the equation

$$\text{RSD } (\%) = \frac{\text{SD} \times 100}{\bar{x}} \quad (1)$$

where $\bar{x}$ is the mean and SD is the standard deviation, calculated by

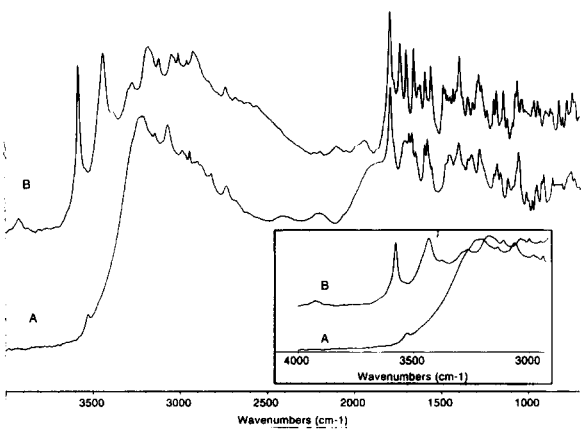


Fig. 2. Qualitative DR mid-IR spectra of cefepime·2HCl: (A) monohydrate; (B) dihydrate; (inset) overlay in spectral region of quantitation.

$$SD = \sqrt{\frac{\sum_{1}^{n}(x_i - \bar{x})^2}{n-1}} \quad (2)$$

and x_i is the measured value and n is the number of results to calculate the mean.

3. Results and discussion

3.1. Characterization

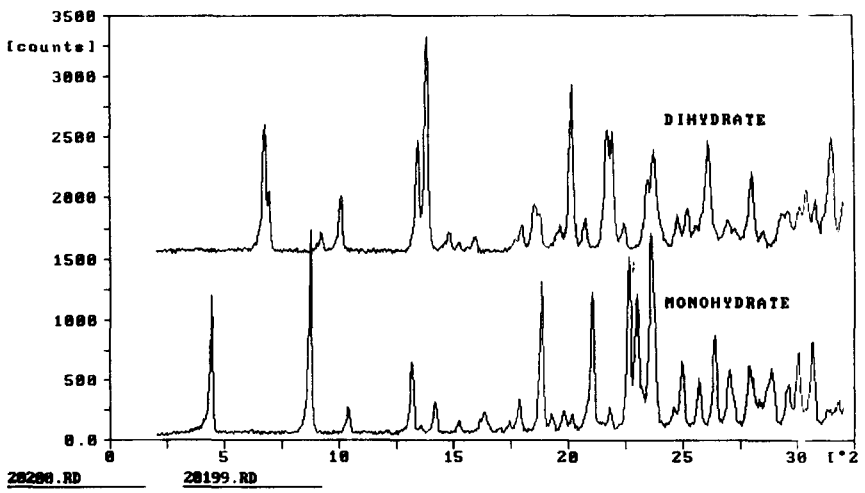
The qualitative diffuse reflectance mid-IR spectrum of cefepime·2HCl monohydrate is shown in Fig. 2A. A single, sharp, absorption band at 3529 cm^{-1} is assigned to the OH stretching mode of the monohydrate functional group. In contrast, the qualitative DR mid-IR spectrum of the dihydrate material (Fig. 2B) displays two distinct bands at 3574 and 3432 cm^{-1}. These two bands correspond to the two distinct dihydrate OH stretching modes. The significant IR spectral differences between the mono- and dihydrate materials in this spectral region (Fig. 2, inset) allow for the quantitation of the dihydrate material in batches of monohydrate. The 3574 cm^{-1} band of the dihydrate material was used for quantitation of this material based on Beer's law [19].

The XRD patterns for the pure cefepime·2HCl monohydrate and dihydrate forms are given in Fig. 3. Two distinctly different X-ray diffraction patterns were measured, signifying two different crystal structures of cefepime·2HCl hydrates. In the region of 12–15° 2θ (Fig. 3B), minimal monohydrate diffraction was observed as compared with the diagnostic dihydrate peak (13.7° 2θ) used for quantitation. Although some monohydrate diffraction is observed in this region (which is most likely due to the background), computer peak fitting of the peak heights minimized any error.

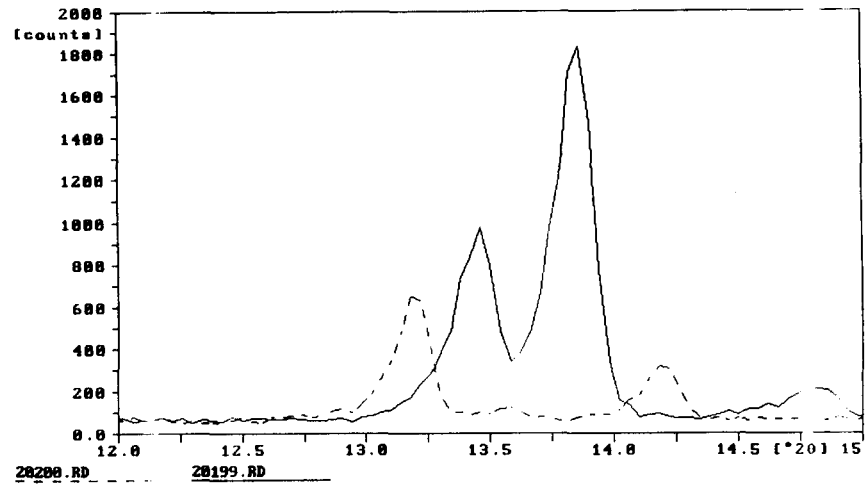
3.2. Sample preparation

Preliminary experiments involving the dry mixing of the two cefepime·2HCl hydrates resulted in inhomogeneous samples. Dry mixing was attempted with a mortar and pestle, a micromill and a Wig-L-Bug. All three techniques gave very poor results. One reason for this problem was the different particle morphologies of the two materials. The monohydrate consists of flat, plate-like structures, as shown in Fig. 4A, whereas the dihydrate material consists of long, thin, needle-like particles, as shown in Fig. 4B. Because the particle morphologies of the two materials are so different, they did not easily mix when dry. Milling experiments did not change the particle sizes sufficiently to influence the mixing properties. Therefore, another sample mixing procedure was investigated.

It was determined that both the mono- and dihydrate materials were insoluble in acetone and therefore an acetone slurry procedure was investigated for preparing homogeneous mixtures. IR and XRD data were compared for pure material "as received" and subjected to the acetone slurry technique to rule out possible crystallographic or chemical transformation during the sample preparation. A single batch of material which contained both monohydrate and dihydrate forms was run as a test lot. Virtually no IR spectral differences or XRD differences were noted between the two samples. The acetone slurry technique does not



(a)



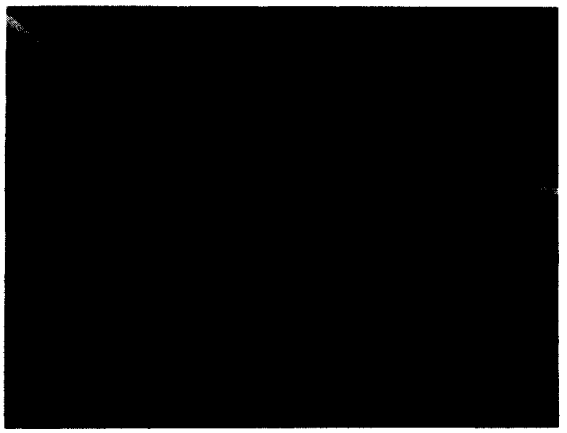
(b)

Fig. 3. X-ray powder diffraction pattern of cefepime·2HCl: (A) monohydrate and dihydrate form; (B) overlay of monohydrate (- - -) and dihydrate (——) in the region 12–15° 2θ.

appear to affect either hydrate significantly. Homogeneous mixing of the solids was achieved using the acetone slurry preparation, as evident in Fig. 4C for the 14.75% dihydrate in monohydrate sample.

Owing to the particle size dependence of the IR and XRD techniques, the particle size distribution resulting from the slurry technique was also investigated. The particle size distributions of three slurried samples were determined using vibrated sieves, and the results are summarized in Table 1. The majority of the particles were found within the 30–60 mesh (595–250 μm) particle size range, while >92% of particles were found within the 30–120 mesh (590–125 μm) range. The minor percentage of particles outside this range (fines and large particles which totaled 8% of total particles) were not used in assay development. The specific particle size range 590–125 μm was required for all samples analyzed by this method, since the calibration samples were prepared in this range. It appeared that samples prepared from the acetone slurry technique resulted in similar particle size distributions. It should be noted that if

(a)



(b)

(c)

Fig. 4. SEM photographs of (A) cefepime·2HCl monohydrate, (B) cefepime·2HCl dihydrate and (C) 14.75% (w/w) dihydrate sample after acetone slurry preparation (all magnifications × 1000).

samples were sieved prior to submission and the particle size distribution did not match that found in Table 1, errors would result in the analysis. These errors would arise from the particle size dependence of the IR and XRD techniques. Variation of peak intensities in the IR spectra are manifested by larger or smaller particles which affects the pathlength (number of diffuse reflections) of IR energy through the sample. In the case of XRD, small particles will minimize preferential orientation effects, thus reducing variations in peak intensities.

3.3. DR mid-IR analysis

After preparation of the calibration, validation and reproducibility samples, DR mid-IR spectra were acquired for each sample. Quantitative analysis of the spectral response for dihydrate content (3574 cm^{-1} absorption band) was performed by spectral integration. This method consisted of setting the spectral limits for integration at 3587 and 3557 cm^{-1}, applying a baseline correction factor and electronically integrating the peak area. Ten calibration samples were utilized to generate the calibration curve shown in Fig. 5. The line was linear up to a concentration of 8.0%. The extremely small y-intercept is indicative that the assay is under good control. During the course of method development, curve fitting and subsequent analytical integration of the fitted curve were attempted to measure the peak areas of the mono- and dihydrate peaks. Unfortunately, the utilization of resolution-enhancement techniques and subsequent Lorentzian and/or Gaussian curve-fitting techniques did not simulate the spectral lineshapes sufficiently.

Validation of the calibration curve was performed by independently examining four different concentration samples. The validation data (correlation curve) are shown in Fig. 6. The fitted slope value of 1.001 and the extremely small y-intercept (-0.0043) again indicate a fairly rugged assay. Although the validation results show excellent correlation between input and calculated concentrations, assay errors can be introduced by a combination of sample packing, sample mixing and sample positioning factors.

Table 1
Particle size distributions (percentage retained) of samples prepared by the acetone slurry technique

Concentration (%, w/w)	30 mesh	40 mesh	60 mesh	80 mesh	120 mesh
1.30	7.6	27.4	46.3	7.4	5.6
5.63	32.3	17.7	30.1	7.1	5.2
11.29	23.8	24.1	35.3	12.9	1.2

The minimum quantifiable level (MQL) is determined from multiple measurements of the spectral response of a concentration approximating the MQL of the assay. Based upon previous crystal form assays developed within the laboratory, an MQL of 5% (w/w) was estimated. Therefore, the MQL was determined by measuring the IR spectral response for a single 5.86% (w/w) sample six different times and utilizing Eq. (3). Based upon a standard deviation of 0.0027 using Eq. (2), an MQL of 0.4% (w/w) was calculated:

$$\mathrm{MQL} = \frac{10 \times \mathrm{SD}}{\text{Slope of calibration curve}}$$

$$= \frac{10 \times 0.0027}{0.0706} = 0.4\% \qquad (3)$$

The limit of detection (LD) was calculated by multiplying the standard deviation by three and then dividing by the slope of the calibration curve [20]. Through this method of calculation, the LD was found to be 0.1% (w/w). The calculated LD and MQL did not agree with experimental studies that were performed. A series of low-concentration samples ($< 0.5\%$, w/w) were analyzed and, by observation of the spectral response at 3574 cm^{-1}, the LD was estimated to be 0.3% (w/w). By analogy, the MQL would then be approximately three times the LD, or approximately 1% (w/w).

As with any reflectance and/or surface-dependent technique such as DR mid-IR analysis, particle size is a major consideration in sample preparation [17]. The slurry technique used for sample preparation fulfilled two tasks: (a) homogeneous mixing of the mono- and dihydrate materials and (b) a consistent particle size distribution for the various cefepime·2HCl mixtures. All samples displayed particle size distributions limited between 125 and 590 μm. Since all calibration, validation and reproducibility samples were prepared within this specific particle size range, any samples in which the concentration is to be predicted by this calibration curve must fall within this range.

3.4. X-ray powder diffraction analysis

The particle size of cefepime·2HCl material used in this X-ray powder diffraction study was not optimized for analysis. A theoretical particle size (t_{max}) can be calculated for the sample when the linear absorption coefficient (μ) is known, using Eq. 4 [18]:

$$t_{max} = \frac{1}{100\mu} \qquad (4)$$

The linear absorption coefficient is calculated from the mass absorption coefficient (MAC) and the density of the material (ρ) [18]:

$$\mathrm{MAC} = \frac{\mu}{\rho} \qquad (5)$$

The mass absorption coefficient corrects for the natural absorption of X-rays by the sample. Based on the MAC and true density values given in Table 2 for the two pure materials, a theoretical particle size of 2.4 μm was calculated for the monohydrate and 2.6 μm for the dihydrate. Obtaining particle sized in this range was not possible with the equipment available. However, owing to the surface-sensitive nature of the technique, it was important to regulate the particle size in some way. The sieving specification was one way to standardize the samples and reduce error.

A straight line model was used for the calculation of the calibration constant:

$$C_i = B'_i I_i \qquad (6)$$

where C_i = concentration of analytical form i, B_i = calibration constant of form i, I_i = net intensity of form i, $B'_i = B_i \times \mathrm{MAC}$ and MAC = mass absorption coefficient of sample mixture. A straight line calibration model can be used if the MAC is a constant or if the concentration range is so small that the MAC is practically constant.

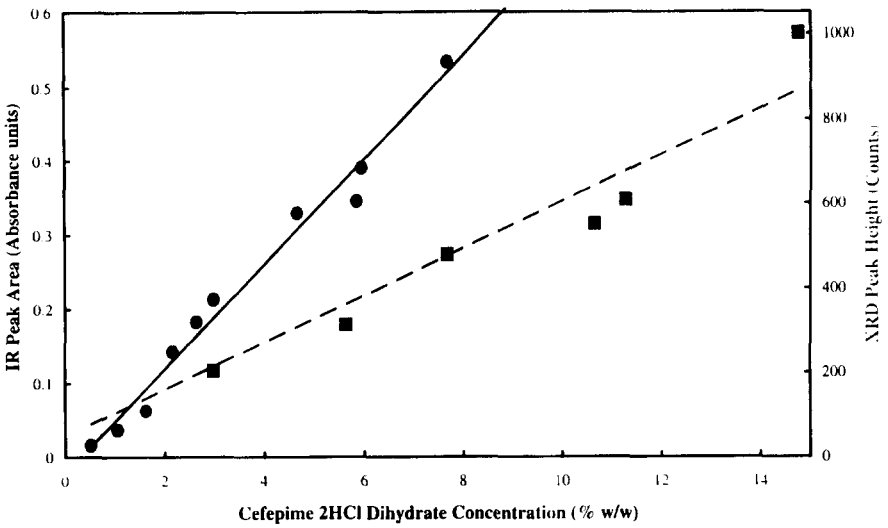


Fig. 5. Calibration curves for the determination of dihydrate content in cefepime·2HCl monohydrate by (●) IR spectroscopy and (■) XRD analysis.

The MAC values calculated for the calibration samples were 28.47, 28.46, 28.44, 28.43, 28.42, and 28.40 for the 2.98, 5.63, 7.68, 10.66, 11.29, and 14.75% (w/w) dihydrate in monohydrate samples, respectively. The MAC values were found to be almost constant, and therefore, the use of the straight line model is valid. The calibration curve for cefepime·2HCl dihydrate form is given in Fig. 5. A calibration constant of 1.7×10^{-2} was calculated from the data. The calibration constant was multiplied by the peak intensity of an unknown sample to determine the concentration of the sample. The validation (correlation) curve for the three samples is presented in Fig. 6. The instrumental reproducibility was measured in this study to calculate the MQL. This was calculated by analyzing a 4.67% dihydrate sample 10 times without removing it from the instrument. An RSD of 5.3% was calculated from the data. This value represents the short-term intensity drift of the instrument and analysis errors in the peak fitting routine.

Analogous to the IR study, the XRD MQL was calculated from Eq. (3). Using a standard deviation of 15.7 and a slope of 62.9, an MQL of 2.5% dihydrate was calculated for this X-ray powder diffraction method. The LD was also calculated in identical fashion to the IR value. Using a standard deviation of 15.7 and a slope of 62.9, an LD of 0.75% (w/w) dihydrate was calculated. This value is similar to the lower limit of detection determined by evaluating decreasing amounts of dihydrate as prepared standards. The dihydrate peak was observed in a sample containing 1.05% dihydrate.

3.5. Assay error evaluation

Various sources of error may be introduced into any DR mid-IR or XRD assay. An overall assay error was determined for each technique by analyzing one sample 10 different times by the IR method and four different times by the XRD method. Based on the IR spectral response, an overall RSD of 12.7% was calculated, whereas an RSD of only 7.8% was determined for the XRD assay. These overall RSDs are actually a combination of errors introduced into the assay by factors such as instrument precision, day-to-day reproducibility, sample positioning, sample packing and sample mixing. Contributions of these individual errors to the overall RSD for each method was estimated and are summarized below:

(1) Instrument reproducibility: this source of error was investigated to determine variability in the instrumentation such as source and detector variance.

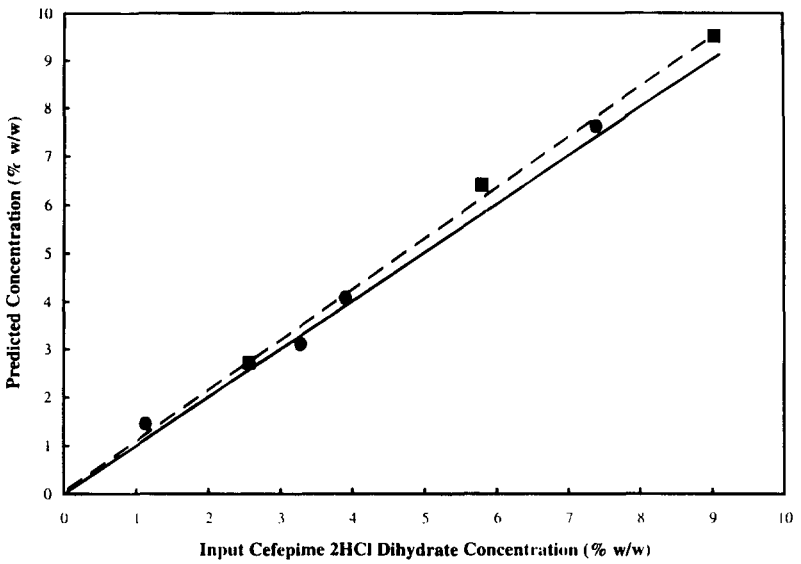


Fig. 6. Correlation curves for the determination of dihydrate content in cefepime·2HCl monohydrate by (●-●) IR spectroscopy and (■—■) XRD analysis.

(2) Day-to-day reproducibility: variability in instrument response on a daily basis was monitored over 4 and 6 days on the IR and XRD instruments, respectively.
(3) Sample positioning: the effect of the position of the sample cup in the DR mid-IR accessory unit or the XRD autosampler was determined.
(4) Sample packing: variation due to crystal orientation was investigated by repacking the same mixture into a DR IR sample cup or XRD sample holder 10 different times.
(5) Sample mixing: variation of sample mixing was determined by preparing a single mixture of sample by the slurry technique and analyzing.

A summary of the individual error contributions for both techniques is given in Table 3. From a review of the various sources of error in these methods, it should be noted that the sample positioning error was a factor in the calculation of any error due to sample packing or sample mixing. Analogously, sample packing contributed to error in the sample mixing study. Unfortunately, the sample packing and sample positioning error contribution in the mixing study cannot be eliminated. The combination of these errors explain the RSDs calculated for both the IR and XRD methods.

3.6. Cross validation

In any multi-disciplinary approach to crystal form characterization and quantitation, numerous physical analytical techniques must be used and

Table 2
Mass absorption coefficient values, density and calculated particle size values for cefepime·2HCl monohydrate and dihydrate materials

Parameter	Monohydrate	Dihydrate
Mass absorption coefficient (MAC)	28.49	27.92
True density (g ml^{-1})	1.46	1.40
Calculated particle size (μm)	2.4	2.6

Table 3
Estimation of assay errors (%)

Source of error	IR	XRD
Instrument reproducibility	0.7 ($n = 6$)	5.3 ($n = 10$)
Day-to-day reproducibility	4.2 ($n = 4$)	6.0 ($n = 6$)
Sample positioning	6.1 ($n = 10$)	6.9 ($n = 10$)
Sample packing	7.3 ($n = 10$)	7.6 ($n = 10$)
Sample homogeneity	8.7 ($n = 10$)	6.2 ($n = 10$)
Overall RSD (%)	12.7	7.8

Table 4
Comparison of IR and XRD experimentally predicted concentrations for five known mixtures

Physically prepared dihydrate concentration (%, w/w)	DR mid-IR experimentally predicted concentration (%, w/w)	XRD experimentally predicted concentration (%, w/w)
1.2	0.8	<MQL
3.0	2.7	3.3
3.8	3.1	3.8
5.2	5.1	4.9
7.5	6.3	7.5

subsequent results compared. In this study, a series of five different mixtures of mono- and dihydrate material were physically prepared and sampled by both IR and XRD and the results compared. Table 4 lists the physically prepared mixture concentrations and the experimentally predicted concentrations via the IR and XRD methods. In each form of analysis, the experimentally predicted amount corresponds relatively well to the known concentration and, more importantly, there is a consistent prediction of concentration when one form of analysis is compared with the other. Based on these results, it appears that either form of analysis is valid and the use of one technique over the other depends on the detection limit required and the availability of equipment.

3.7. Effect of particle size on quantitation

It is widely understood that particle size has a pronounced effect on any quantitative DR mid-IR or XRD assay [17,18]. This argument was especially true for method development concerning cefepime·2HCl. During the course of IR method development, a 7.56% (w/w) dihydrate in monohydrate sample was passed through the required series of sieves. Approximately 70% of the material was retained on the 60-mesh screen. Only the 60-mesh screen-retained material was sampled via the DR mid-IR assay. After evaluation of the spectrum, the experimentally predicted amount of dihydrate material was 29.5% greater than the theoretical amount. Once the retained material on each sieve has been recombined and assayed again, an error of 10% existed between the physically prepared and experimentally predicted concentrations. It is readily apparent that the DR mid-IR assay is particle size specific ($125 < x < 590$ μm), and only samples which display this particle size range may be sampled by this technique.

The effect of particle size and morphology on the X-ray powder patterns of the cefepime·2HCl forms was investigated using a sample passed through a 100-mesh sieve. Qualitative powder patterns collected for the different particle size fractions of the dihydrate form are presented in Fig. 7. Obvious differences are noted between the powder patterns. In the region of quantitation (12–15° 2θ), the smaller particle size results in a substantial decrease in peak intensity. Other regions of the XRD pattern show increases in peak height for the smaller particles. This can be explained by possible preferential orientation of the larger particles based on the particle morphology. As seen in the scanning electron micrographs, the dihydrate exists as needles, which is known to present preferential orientation problems. When the particle size of the needles is decreased, the needles will be broken apart and some crystallographic planes will be observed less, resulting in a decrease in peak height, whereas some planes will be observed more, resulting in an increase in peak height. By decreasing the particle size and reduc-

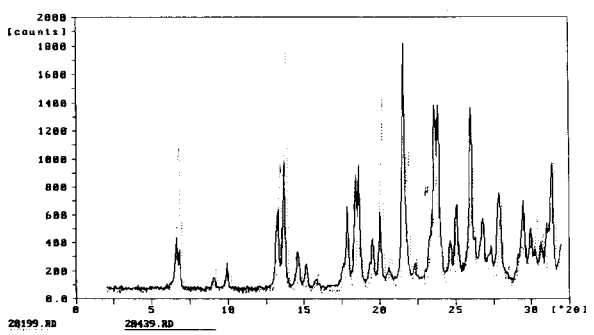


Fig. 7. Qualitative XRD powder patterns of cefepime·2HCl dihydrate as received (· · ·) and passed through 100 mesh sieve (——). A slight 2θ shift occurs between the two diffraction patterns and is due to variations in sample height.

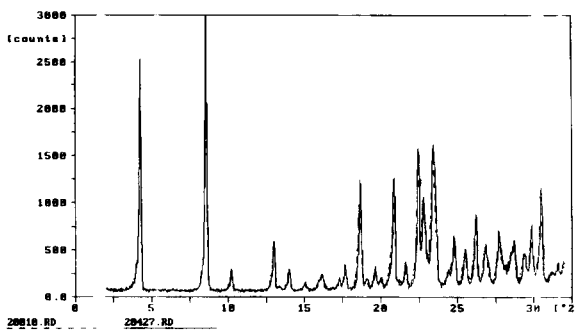


Fig. 8. Qualitative XRD powder patterns of cefepime·2HCl monohydrate as received (- - -) and passed through a 100-mesh sieve (——).

ing preferential orientation, a more representative powder pattern is obtained for the dihydrate form. This variation in peak height due to particle size is not as pronounced for the monohydrate form, as shown in Fig. 8. The plate-like morphology of this form is not as prone to the preferential orientation problems associated with the dihydrate needles. Further discussions of preferential orientation can be found elsewhere [18].

The effect of particle size in quantitative XRD analysis was demonstrated for the dihydrate form. Using identical methodology as described previously for samples within the 125–590 μm range, a three-point calibration curve was produced for material passed through a 100-mesh screen. The calibration constant calculated from this curve is 3.3×10^{-2}, which is twice as large as that calculated previously for the larger particles. Using one calibration constant for samples of various particle sizes when calculating concentration can introduce significant errors in the quantitation. From these data it is evident that particle size plays an important role in the quantitation of crystalline samples via XRD.

3.8. Method improvements

To improve the IR and XRD quantitative results, several factors could be optimized: (a) a more specific particle size range would need to be specified, and a convenient way of measuring or insuring that range would need to be developed; the particle size of a sample can cause up to a 20% error in the quantitative data collected [18] and therefore should be closely monitored; (b) sample packing into the IR sample cup or XRD holder also needs to be controlled; a sample weight was specified in the XRD procedure, but the use of constant pressure for packing the sample into either the IR cup or the XRD holder was not investigated; (c) for XRD, the sample surface needs to be as smooth as possible and guidelines would be needed to reject samples with excessive surface roughness; and (d) XRD samples could also be pressed into pellets to obtain surface smoothness and increase the signal. Again, a specific sample weight and pressure would be used for all sample preparation. By incorporating these improvements into the quantitative analyses, better precision and reproducibility would be obtained.

4. Conclusions

A quantitative diffuse reflectance mid-infrared and X-ray powder diffraction procedure has been developed to determine the amount of cefepime·2HCl dihydrate in cefepime·2HCl monohydrate material. In order to achieve homogeneous calibration and validation samples, a prerequisite for any solid-state assay, an acetone slurry sample preparation was used. The diffuse reflectance mid-IR assay displayed a working range of 1.0–8.0% (w/w) dihydrate in monohydrate. An MQL of 1.0% (w/w) dihydrate and an LD of 0.3% (w/w) dihydrate were estimated with an overall RSD of 12.7%. The powder X-ray diffraction assay displayed a working range of 2.5–15% (w/w) dihydrate in monohydrate with an MQL of 2.5% and an LD of 0.75%. A lower RSD of 7.8% was demonstrated by the XRD assay. Each assay is only valid for samples which exist within a particle size range of 125–590 μm. Evaluation of the errors associated with each assay indicate that although the acetone slurry sample preparation technique provided homogeneous mixtures (as compared with dry mixing), manipulation of the sample (positioning, packing and/or mixing) is the largest source of error.

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