Development and validation of analytical methodology for near-infrared conformance testing of pharmaceutical intermediates

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Abstract

An approach was developed to create and validate analytical methods to perform near-infrared (NIR) conformance testing on two isolated intermediates used in the manufacture of loracarbef monohydrate bulk drug substance. Method calibration sets were developed from second-derivative NIR reflectance spectra for 30 representative batches of each intermediate. In conformance testing, second-derivative NIR spectra for samples from newly manufactured batches are compared with the calibration set. If the new spectrum is not statistically different to the average of the calibration set, the sample passes the conformance test. Using authentic batch samples of typical and low-potency lots, the methods were validated for accuracy, selectivity, ruggedness and repeatability of the methods.

Keywords: Pharmaceutical analysis; Near-infrared spectroscopy; Method validation; Loracarbef; Conformity index; Conformance testing; Wavelength distance method

1. Introduction

Near-infrared reflectance spectroscopy (NIRS) has been gaining widespread acceptance in the pharmaceutical industry as a rapid and sensitive analytical technique [1,2]. Applications include identification testing, moisture content determination, and even non-destructive whole tablet identification and assay [3,4]. Sample absorbances in the near-infrared (NIR) region arise from combinations and harmonics of heteroatom (R–H, where R = O, N, C, etc.) resonances in the mid-

infrared region. Because of this, NIR absorbances are comparatively weak. This, combined with reflectance analysis, allows pharmaceutical samples to be analyzed with little or no sample preparation. Not only does this save laboratory analyst time, but also preparation steps which contribute to measurement variability are eliminated. Differences in particle size and crystal form, changes in levels of impurities or the presence of minor contaminants can all affect the NIR spectrum of a sample. This, together with the speed of an analysis, makes NIRS an ideal identification tool [5,6] that has been recognized as a powerful method of

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"fingerprinting" pharmaceuticals [7]. Rugged NIR instruments equipped with fiber-optic sample probes and user-friendly software are now readily available. These instruments allow analyses such as raw material identification to be done in the manufacturing plant by personnel with little laboratory training.

Current economic forces are placing pressure on the pharmaceutical industry to become more cost competitive. At the same time, increasing expectations and regulations of agencies such as the Food and Drug Administration and similar regulatory agencies around the world have increased the costs of drug development and manufacture. One important way to decrease manufacturing costs is to produce quality products consistently, with a process that is in statistical control and produces a product that is capable of meeting quality specifications minimizes factory losses.

A common practice is for samples of pharmaceutical intermediates isolated during manufacture of a bulk drug substance to be assayed in the quality control laboratory. Specifications often include a potency determination, typically by high-performance liquid chromatography (HPLC); an identification test, typically by HPLC or infrared (IR) spectroscopy; and residual volatiles determination, typically by Karl Fischer titration for water or by weight loss on drying for organic solvents. The test method is often the major source of variation in potency results for intermediates produced by processes that are in statistical control and compliant with specifications. When this is the case, a common practice is for all batches to be assigned a standard potency (e.g. the average potency of 30 or more lots). In many cases, each batch is still tested to assure conformance to an established set of quality standards as measured by one or more analytical tests. Since the product quality is consistent, this testing is done to identify a small percentage of batches which fall outside specifications due to special causes. For such consistent processes, NIR conformance testing has been developed as an alternative strategy [8].

In conformance testing, a calibration set of NIR spectra are recorded from a representative collection of approved batches. For the conformance test, an NIR spectrum of a sample from a new batch is measured and compared with the calibration set. If the new sample spectrum is not statistically different to the spectra from the approved lots, the sample passes the conformance test. The implication is that the new batch conforms to the same quality standards as the calibration batches. In this sense, the conformance test is a more stringent comparison than a simple identification test, and may sometimes be referred to as qualification. This alternative strategy greatly reduces the non-value-added tasks involved in testing acceptable lots, and yet will still identify batches outside specifications due to special causes. This approach has been used by the pharmaceutical manufacturer Gist-Brocades (Delft, The Netherlands) to replace the compendial tests for potency, water content and identification of bulk ampicillin trihydrate as reported by Plugge and Van Der Vlies [9].

The statistical method for the conformance test used by Plugge and Van Der Vlies has been called the wavelength distance method [10]. In this method, an average NIR spectrum for the calibration set is calculated, along with a standard deviation spectrum (the classical standard deviation, at each wavelength, computed over the training set). The spectra are typically converted to first- or second-derivative spectra before calculations in order to compensate for baseline offset differences between the individual primary reflectance spectra. Other treatments of the data, such as digital smoothing, may be included in this preprocessing stage. In the comparison of the new spectrum with the calibration set, its difference from the average spectrum is calculated as a residual spectrum of z-scores (difference normalized by the standard deviation) [10]. If the magnitude of each individual z-score is smaller than a predefined threshold, the new spectrum is judged to be not statistically different to the calibration set, and the sample passes the conformance test. If at least one z-score exceeds the threshold, the spectrum is judged to be statistically significantly different, and the sample fails the conformance test. The largest z-score is reported as a measure of the distance of the new spectrum from the calibration
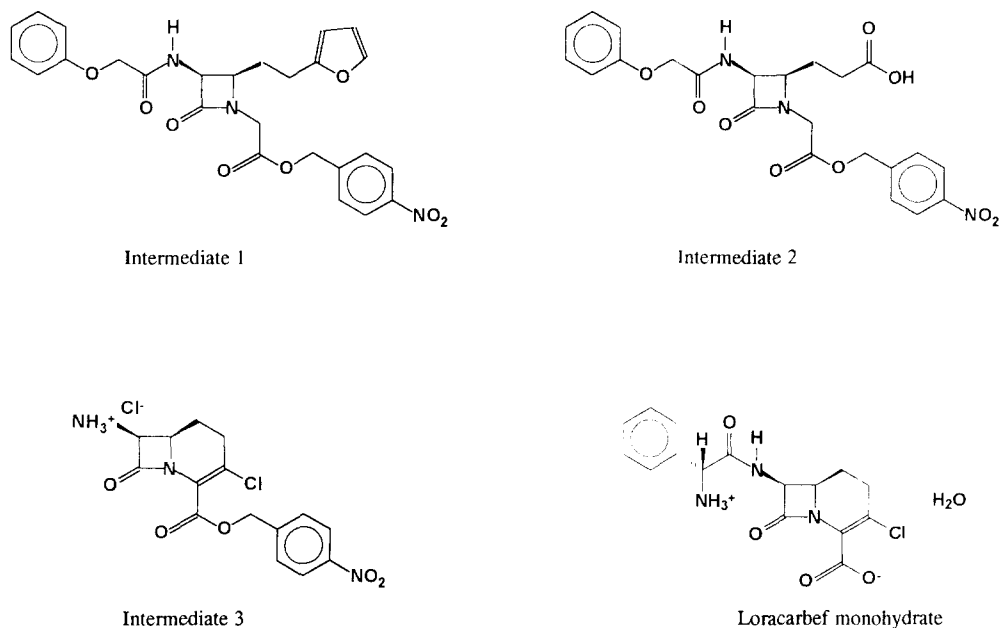
Fig. 1. Structures of loracarbef monohydrate and synthetic intermediates.

set, and may also be referred to as the conformity index (CI) [8]. The appropriate threshold value for comparison is a function of the size of the calibration set and the number of comparisons made (individual points in the NIR spectrum). For example, using the Student's *t* distribution and binomial probability theory, Gemperline and Boyer

Table 1
Results for accuracy of NIR conformance test on intermediate 3

Sample identification	HPLC potency (%)	NIR conformance	CI
13-A	86.6	Pass	3.32
13-B	88.3	Pass	3.48
13-C	84.5	Fail	5.78
13-D	80.6	Fail	8.04
13-E[a]	74.2	Fail	10.2
13-F[a]	88.8	Pass	3.59
13-G[a]	89.3	Fail	5.05
13-H[a]	89.7	Fail	5.00

[a] Samples labeled 13-E, 13-F, 13-G and 13-H were prepared in the laboratory by filtering a process slurry sample and washing with 0% (unwashed), 25%, 50% and 75%, respectively, of the standard dichloromethane wash volume, scaled down to laboratory proportions.

[10] calculated that a threshold of 5.0 standard deviations will result in a 98.2% confidence level for a calibration set consisting of 30 spectra with 700 data points.

In a pharmaceutical quality control laboratory, analytical method validation is required under current good manufacturing practices (cGMPs) [11]. The United States Pharmacopeia (USP) provides some guidance for the validation of analytical test methods [12]. Unfortunately, the USP does not suggest an approach for validation of the relatively new NIR chemometric methodologies. The chemical literature also provides scant help. The intent of this paper is to share a standard approach, developed in our laboratory, for the validation of NIR conformance test methodology. Use of this approach is demonstrated in the validation of conformance test methods for two intermediates used in the manufacture of loracarbef bulk drug substance.

2. Experimental

All NIR reflectance spectra were collected using a Bio-Rad FTS40A Fourier transform NIR in-

Table 2
Potency, total related substances and conformity indices for intermediate 1 lots

Sample identification	Potency (HPLC)(%)	Total related substances (%)	CI			NIR conformance
			Result 1	Result 2	Average	
I1-A	98.3	1.1	4.41	3.36	3.89	Pass
I1-B	98.9	0.8	3.56	3.65	3.61	Pass
I1-C	96.5	2.6	5.02	4.03	4.53	Fail/Pass
I1-D	94.9	3.7	5.83	6.54	6.19	Fail
I1-E	93.5	5.7	8.49	9.64	9.07	Fail
I1-F	80.9	18.0	28.55	29.88	29.22	Fail
I1-G[a]	99.3	–	–	–	–	

[a] Utilized in ruggedness study (see text).

strument (Bio-Rad Laboratories, Cambridge, MA, USA). The spectral range covered was 1333–2500 nm (7500–4000 cm^{-1}). The scan speed was 5 kHz, the delay was 3 s and the low-pass filter was set to 4.5 kHz. For each spectrum, 64 individual scans were collected (taking approximately 60 s) and summed for transformation. The aperture was open, the collect sensitivity was 1 and the resolution was 8 cm^{-1} with one zero-fill, resulting in an NIR spectrum consisting of 910 digital wavelength–absorbance pairs.

A crescent model 3110B WIG-L-BUG sample mull was used for grinding the reference and/or samples. Certified ACS-grade potassium bromide (KBr) obtained from Fisher Scientific was used

Fig. 2. Plot of conformity index vs. HPLC Potency for samples used in the validation of NIR conformance test accuracy for intermediate 1.

for recording the reference spectrum. To control the consistency of the grinding, 400 ± 10 mg of sample or KBr was placed in a disposable polycarbonate cup with three 4 mm glass beads and mulled for 60 s. The ground sample or KBr was then gently packed into a 6 mm i.d. × 2 mm deep open sample cup supplied by Bio-Rad for use with the diffuse reflectance accessory. The KBr reference and/or samples were allowed to purge with 30 standard cubic feed per hour (scfh) of dry nitrogen for approximately 60 s after placement in the instrument sample chamber, to remove water vapor from the sample chamber. The purge time for the samples was matched to the reference in order to background subtract correctly the response of any residual water vapor.

Samples of the intermediates were obtained from previously tested quality control laboratory samples of batches. The structures of loracarbef and the intermediates used in the study are shown in Fig. 1. Intermediate 1 is 1-azetidineacetic acid, 2-[2-(2-furanyl)ethyl]-4-oxo-3-[(phenoxyacetyl)amino]-, (4-nitrophenyl)methyl ester (2R-cis)-, CAS No. 131533-61-4; intermediate 2 is 2-azetidinepropanoic acid, 1-[2-[(4-nitrophenyl)methoxy]-2-oxoethyl]-4-oxo-3-[(phenoxy-acetyl)amino]-, (2R-cis)-, CAS No. 131533-72-7; intermediate 3 is 1-Azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-amino-3-chloro-8-oxo-, (4-nitrophenyl)methyl ester, monohydrochloride (6R trans)-, CAS No. 123932-46-7; and loracarbef monohydrate is 1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-
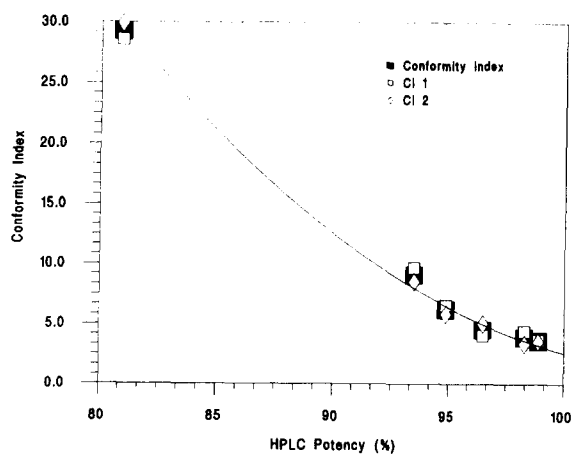
Table 3
Specificity of the intermediate 1 NIR conformance method

Description	NIR conformance	CI
Intermediate 2	Fail	139.4
Intermediate 3	Fail	103.6
Intermediate 4	Fail	30.6
Intermediate 5	Fail	50.2
Loracarbef monohydrate [a]	Fail	58.8
Loracarbef monohydrate [b]	Fail	62.5

[a] Capsul grade.
[b] Oral pediatric grade.

chloro-8-oxo, monohydrate, {6R-[6α, 7β (R*)]}-, CAS No. 121961-22-6. Intermediate 4 is a dimethylformamide disolvate crystal form of loracarbef, and intermediate 5 is an ethyl alcohol solvate of loracarbef. The structures of intermediates 4 and 5 are not shown, since they differ from loracarbef only in solvents of solvation versus water of hydration. Samples of intermediate 3 were mulled before analysis, owing to particle size differences from sample to sample and to homogenize samples occasionally containing large aggregates. Samples of the other intermediates were not mulled.

Calibration of the NIR conformance methods was accomplished by recording single NIR spectra for samples from 30 typical batches of each intermediate. Normal variation was introduced into the calibration sets by collecting the spectra

Table 4
Specificity of the intermediate 3 NIR conformance method

Description	NIR conformance	CI
Intermediate 1	Fail	120.0
Intermediate 2	Fail	132.6
Intermediate 4	Fail	35.1
Intermediate 5	Fail	58.8
Loracarbef monohydrate [a]	Fail	56.5
Loracarbef monohydrate [b]	Fail	59.8

[a] Capsule grade.
[b] Oral pediatric grade.

over several different days by multiple analysts. Before inclusion in the calibration sets, the assay history of the samples was examined to exclude any batches potentially lower in potency than typical. This was done by first examining the related substances assay data for unusual batches. HPLC assay data was examined by creating a control chart (X-chart) of the calibration lot potencies and examining the chart for evidence of batches with unusual potency due to special causes (out-of-control points). The potency of intermedidate 1 centered around 98.8% with a standard deviation of 0.72%, resulting in a three standard deviation range of 96.7–101.0%. The potencies of all calibration batches for intermediate 1 were contained in this interval. The average potency for batches of intermediate 3 was 88.4% with a standard deviation of 0.86%, corresponding to a three standard deviation range of 85.8–91.0% for intermediate 3. The potencies of all calibration batches for intermediate 3 were contained in this interval. For both of the intermediates, the variation introduced by the HPLC assay method itself (estimated by repeated analysis of a single batch) was greater than the true variation in potency from batch to batch. Thus, for both intermediates, the HPLC potencies of all of the calibration batches were statistically indistinguishable, and are therefore not reported.

Spectra were converted to unsmoothed second-derivative spectra using the Bio-Rad software, and converted to text files. The text files were transferred from the Bio-Rad SPC 3200 computer controlling the FTS40A to an EPSON Equity III+ personal computer. A program written internally using FORTRAN 77 for the MSDOS operating system was used to perform all statistical calculations. The program was validated by cross comparison of calculations with Microsoft Excel. Two separate routines were written. One routine performed method calibration by calculating a calibration matrix of average second-derivative absorbances and standard deviations at each digital point. The second routine performed the conformance test by comparison of the sample second-derivative spectrum with the calibration matrix. A threshold of 5.0 standard deviations was used for all conformance tests. This value was
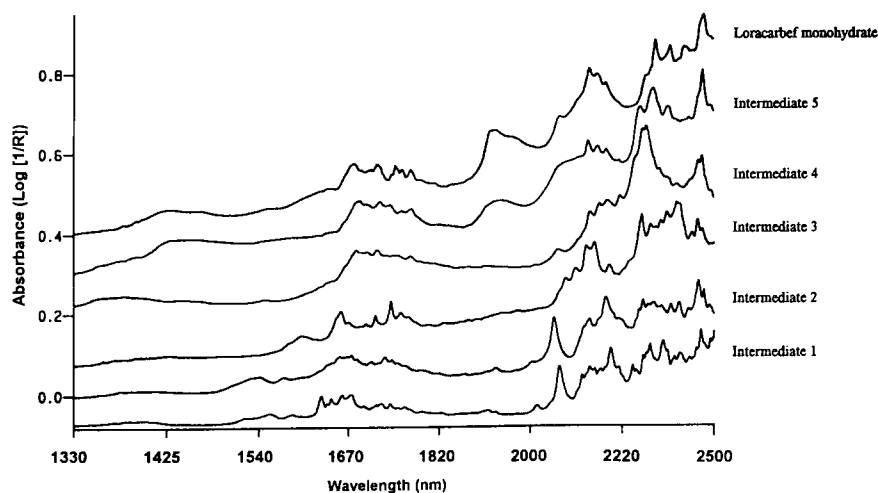
Fig. 3. Near-infrared reflectance spectra for loracarbef monohydrate and synthetic intermediates.

used for consistency with the work of Plugge and Van Der Vlies, and corresponds to a 97.7% confidence level using the calculations reported by Gemperline and Boyer.

3. Results and discussion

Following calibration, the NIRS conformance test methods were validated by a series of experiments to examine accuracy, specificity and ruggedness. Initially a larger spectral range (1100–2500 nm or 10 000–4000 cm^{-1}) was utilized. In the NIR spectra for the intermediates, the region from 1100 to 1333 nm was found to contain little spectral information. That from 1100 to 1333 nm also coincided with a region where the throughput of the optical bench was relatively poor, causing increased background noise. The range was reduced to 1333–2500 nm to improve the signal-to-noise ratio for the data.

3.1. Validation of NIR conformance accuracy

The accuracy of the NIR conformance test for intermediate 3 was evaluated by challenging the method with samples of both acceptable and unacceptable quality intermediate 3. The acceptable samples were from batches which conformed to all assay specifications (batches I3-A and I3-B). A

minimum of two samples of batches typical in potency was felt to be sufficient to demonstrate the accuracy of the method. The ruggedness of the method toward multiple, acceptable batches would be addressed during a trial implementation of the method at a later time. The unacceptable samples were chosen from authentic batches low in potency due to manufacturing deviations. These samples were from batches which assayed below the HPLC potency specfication of not less than (NLT) 85.0% (batches I3-C and I3-D). Further analysis of batch I3-C showed that its average potency was 85.1%. This was an ideal validation test sample, as its potency was different than the process average, and just above the specification limit.

In order to generate additional validation samples, a sample was taken from a typical batch of intermediate 3 during the filtration isolation step, before the filter-cake washing. Four portions of the sample were washed and filtered in the laboratory with different amounts of dichloromethane in an attempt deliberately to produce samples of intermediate 3 with low potency. The samples were allowed to air dry before testing.

Table 1 summarizes the results of the NIR conformance tests performed on the accuracy test samples of intermediate 3. Both samples that passed the initial HPLC assay test passed by NIR conformance. Both samples that failed the initial

Table 5
Ruggedness data for NIR conformance on intermediate 3

Day	Analyst	Sample identification	NIR conformance	CI	Sample identification	NIR conformance	CI
1	A	I3-A	Pass	3.51	I3-C	Fail	8.47
1	A	I3-A	Pass	3.54	I3-C	Fail	6.91
1	B	I3-A	Pass	3.39	I3-C	Fail	7.23
1	B	I3-A	Pass	3.39	I3-C	Fail	8.46
1	B	I3-A	Pass	3.51	I3-C	Fail	6.94
2	A	I3-A	Pass	4.46	I3-C	Fail	8.19
2	A	I3-A	Pass	4.22	I3-C	Fail	8.76
2	A	I3-A	Pass	4.98			
3	B	I3-A	Pass	3.72	I3-C	Fail	9.89
3	B	I3-A	Fail	5.53	I3-C	Fail	8.42
3	B				I3-C	Fail	9.72
Average CI				4.03			8.30
RSD				18.6%			12.6%

HPLC assay test failed by NIR conformance. All of the laboratory-prepared samples failed NIR conformance, except for I3-F (25% wash volume). I3-G, and I3-H did not pass NIR conformance, although their potency assays were above 85.0% and, in fact, perhaps slightly higher than typical batches. This suggests that although reduced wash volumes were used, the laboratory washing procedure may be more efficient than the full-scale process in removing impurities. Since these samples were treated in the laboratory, it was not surprising that they were found to be statistically different by NIR conformance than the batch samples used for calibration of the method. The laboratory procedure for washing and drying these sample batches differed from the manufacturing process in both scale and equipment, and hence could introduce differences in crystal size distribution, for example. NIRS has been shown to be sensitve to such differences, and is in fact reported to be sensitive to manufacturing process changes [7].

In order to determine the effect of residual solvents on the NIR conformance test, a passing batch of the intermediate 3 was spiked with isobutyl alcohol, a solvent used in its manufacture. It was hoped that the NIR conformance test would replace the assay for volatiles by weight loss on drying. The spiking procedure was repeated using dichloromethane, the final wash sol-

vent. Unfortunately, owing to the open design of the sample cup and the volatility of these solvents, the method was found to be insensitive to residual solvents below about 10–20% by weight owing to evaporation during sample preparation and measurement. This result emphasizes the importance of analytical method validation.

The accuracy of the NIR conformance test for intermediate 1 was evaluated by challenging the method with samples of both acceptable and unacceptable quality intermediate 1. Samples from batches passing all quality control assay specifications, and typical in potency, were used as the acceptable samples (I1-A and I1-B). In the case of this intermediate, a number of batches with potencies lower than typical were available (I1-C, I1-D, I1-E and I1-F). Each sample was prepared and tested in duplicate, for this study, to begin examining the repeatability of the conformance test. Table 2 shows the HPLC potencies, related substances assay results and the conformity indices obtained for the samples of intermediate 1 used to validate the accuracy of the NIR conformance test.

The typical batch samples passed the NIR conformance test. Batch samples lower in potency, I1-F and I1-E, failed the NIR conformance test. Batch I1-E was an ideal validation test sample, as its potency was just above the specification limit of NLT 93.0%. The failure of this batch provides
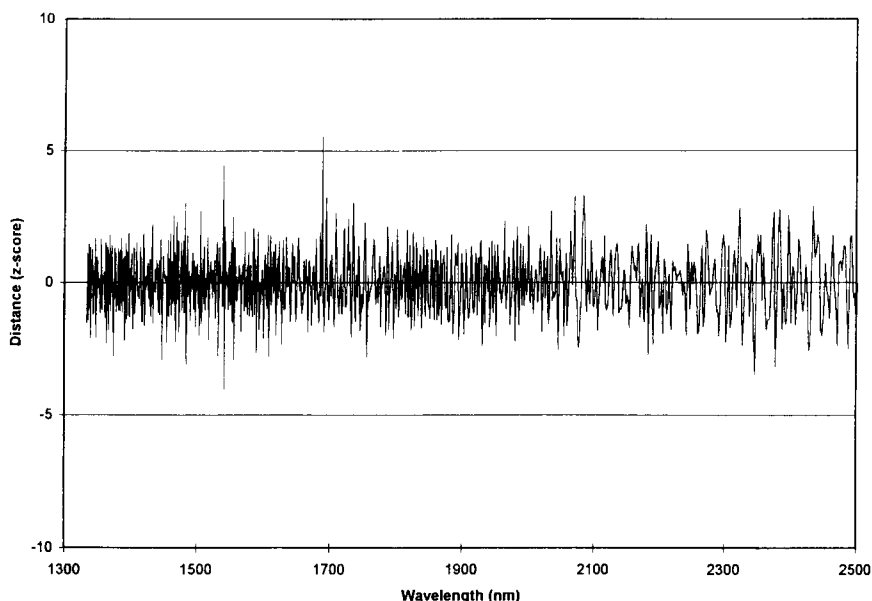
Fig. 4. NIR conformance test for failing preparation of intermediate 3 batch I3-A.

evidence that the NIR test will fail batches falling below the specification limit. Batch I1-D, which was about 3% lower in potency than typical, failed conformance, whereas batch I1-C, which was only about 1–2% lower in potency, passed once and failed once. Fig. 2 shows a plot of the conformity indices vs. the potencies for the intermediate 1 accuracy validation lots. These data demonstrate that the sensitivity of the NIR method to potency differences is about 2–3%, that is, intermediate 1 batches that are more than 2–3% lower in potency than typical will fail conformance, whereas batches which are within 1–2% of typical will pass.

Having demonstrated that samples of batches approaching the potency specification limits for both intermediates failed and that batch samples of typical potency passed conformance, the NIR conformance test methods were considered validated as accurate alternatives to the HPLC potency assays. In both cases, authentic batch samples of the intermediates with potencies just above the specification limits failed conformance. The method was demonstrated to be sensitive to 2–3% potency differences between test samples and samples comprising the calibration set.

3.2. Validation of NIR conformance specificity

In order to validate their specificity as identity tests, the NIR conformance methods were challenged with samples of other substances. Chemically similar samples from other different intermediates in the manufacture of loracarbef and the final bulk drug itself were tested by both NIR conformance methods. Tables 3 and 4 show the results obtained for validation of the specific-

Table 6
Stability of sample preparation for NIR conformance on intermediate 3

Sample identification	Description	NIR conformance	CI
I3-A	Normal preparation	Pass	4.05
	10 min purge	Pass	4.17
	10 min on bench	Pass	3.90
I3-C	Normal preparation	Fail	7.81
	10 min purge	Fail	7.98
	10 min on bench	Fail	8.54

ity of the NIR conformance methods as identification assays for intermediates 1 and 3, respectively.

The results of this study demonstrate excellent specificity of the methods for the respective intermediates. All samples of the different intermediates failed conformance with CIs significantly greater than 5.0. The most similar intermediate, in both cases, was intermediate 4. Fig. 3 shows typical NIR spectra for loracarbef monohydrate and the intermediates studied here. It is interesting that the spectrum for intermediate 4, contains the fewest sharp features. Intermediate 4 is the dimethylformamide disolvate crystal form of loracarbef; the relatively high concentration of this solvent perhaps explains the lack of sharp absorbances. Although this is not well understood, it may be the sharper spectral features, enhanced by the second-derivative math treatment, that provide the most differentiation between the intermediates.

3.3. Validation of NIR conformance ruggedness

Two batch samples of intermediate 3 were used in a study of the ruggedness of the NIR conformance method: batch I3-A (typical in potency) and batch I3-C (lower than typical potency, but just above the specification limit of NLT 85.0%). The ruggedness of the conformance test was determined by independently preparing and assaying the samples 10 times each, by two analysts, on at least two different days. The ruggedness data are shown in Table 5.

Table 5 includes a calculation of the average CI and the relative standard deviation (RSD) for the 10 results. This is reported as a general measure of the reproducibility of the conformance test; however, it is important to note that the CIs are the maximum of a large number of z-scores. These RSDs should not be directly compared with results generated from single determinations (i.e. an HPLC potency assay). All 10 replicates of the lower potency sample (I3-C) failed NIR conformance. Nine out of 10 replicates of the normal potency sample (I3-A) passed conformance. An investigation was made to determine the cause of the false-negative result for sample I3-A. Fig. 4 shows the plot of the z-scores obtained in the

NIR conformance test for this failing sample. The primary NIR reflectance spectrum and the second-derivative spectrum for the failing test were compared with typical spectra of the same sample for passing tests. The single wavelength corresponding to the absorbance causing the failure was found in a spectral region where samples exhibited little absorbance. This suggests that the sample may have failed owing to baseline noise. The noise in this region was found to increase when the lamp energy was attenuated and, in fact, acceptable batch samples could be made to fail by lowering the lamp energy (i.e. by slightly misaligning the lamp). Conversely, higher lamp energies produced NIR spectra with better signal-to-noise ratios. Based on these results, it was realized that sufficient lamp power must be ensured to reduce noise in the NIR spectra.

To investigate ruggedness with respect to sample preparation, crevasses were deliberately introduced in sample preparations. This was found to increase the possibility of a false-negative result: one of two samples prepared in this way failed conformance. To investigate the stability of sample preparations, samples of the passing batch and the failing batch were exposed to laboratory air for 10 min before analysis. Additional preparations of these samples were allowed to remain in the instrument chamber under the NIR beam and dry nitrogen purge for 10 min. Exposure or purge times longer than 10 min were not examined since the intent of the conformity test was to provide a rapid sample analysis. Table 6 shows the results obtained from this experiment. In all cases, the passing sample continued to pass and the failing samples continued to fail, suggesting sample preparations of intermediate 3 were stable to the laboratory environment and extended purging.

To examine the ruggedness of the NIR conformance method in more detail, the study was increased from two to four samples of batches for intermediate 1. Each batch was tested 10 times, by two analysts, on at least two different days. Two batches with typical potency were examined, I1-B and I1-G. The average HPLC potency assay for a typical batch was 98.8%. Two batches with lower potency than typical were examined, I1-C (HPLC potency 96.5%) and I1-D (HPLC potency 94.9%).

Batches 11-B, 11-C and 11-D were the same batches used in the validation of accuracy (see below). The repeatability data are shown in Table 7.

Typical batches passed 19 out of 20 conformance tests; the failing test is shown in Fig. 5. Examination of the wavelength region with the failing z-score indicates that the cause of this failure may be the same as described above for intermediate 3. With a 98% confidence threshold, one would expect about one out of every 50 tests to result in a false-negative, simply due to random noise. A false-negative frequency higher than this could indicate that the calibration set does not sufficiently model the random noise. This would result if the calibration spectra were collected under optimal conditions (i.e. new lamp, careful instrument adjustment), different to the conditions of routine use. This could be corrected either through more careful instrument adjustment on an ongoing basis, or by inclusion of the less optimal spectra in the calibration set. Batch 11-D failed nine out of 10 times, whereas batch 11-C, surprisingly, passed all 10 times. It is important to note that 11-C was chosen for this study, although previous testing (see Table 2) showed this batch to be at the borderline of the discrimination capability of the NIR conformance test. A lower potency batch such as 11-E (HPLC potency 93.5%) could have been selected; however, it was felt that more could be learned by including batch 11-C in the study. These data show that batches having typical potencies should reliably pass 95% of the time. Batches such as 11-C, with potencies only 1–2% lower than typical, will not reliably fail the conformance test. Batches such as 11-D (HPLC potency 94.9%), which are 2–3% lower than typical potency, will fail conformance more consistently. This, along with the accuracy data, supports the contention that batches with potencies approaching the specfication limit of NLT 93.0% will reliably fail the NIR conformance test.

To investigate the stability of sample preparations, samples from three of the four batches of intermediate 1 studied above (11-D, 11-B and 11-G) and a sample from batch 11-E were exam- ined. Batch 11-E replaced batch 11-C, which would be expected to pass NIR conformance. Sample preparations were tested for NIR conformance after (1) a minimum purge time, (2) exposure to laboratory air for 10 min before analysis (with a standard 60 s purge) and (3) remaining in the sample chamber under the NIR beam and dry nitrogen purge for 10 min. Table 8 shows the results of this sample stability study. All preparations of batch 11-G passed conformance and all preparations of 11-E failed conformance, as expected. Two preparations of batch 11-B (typical in potency) failed, and the preparation of batch 11-D purged in the instrument for 10 min unexpectedly passed. The reasons for the failures of the typical batch were unclear, with the failing wavelengths in regions with spectral absorbances evident, versus baseline. These data demonstrate a need for careful control of the preparation and purge time for the intermediate 1 samples. This appears to be more important to control false-negative results and for the discrimination of batches only slightly lower in potency.

As a result of understanding and controlling the impact of factors such as sample purge time, the NIR conformance methods were found to be sufficiently rugged for routine use. The frequency of obtaining a false-negative result for a typical batch was one out of 10 times for intermediate 3 and one out of 30 times for intermediate 1. This frequency was higher than the 2% one would expect for a threshold calculated to correspond to 98% confidence. Adjustments could be made to the calibration sets to provide more ruggedness with respect to regions with low signal-to-noise ratio. Alternatively, these regions could be excluded entirely.

When a sample fails the conformance test, the spectral data should be reviewed to provide some insight into the cause of the failure. If a batch sample consistently fails conformance after instrument problems or errors in technique are corrected, samples of the failing batch should be analyzed by the primary HPLC potency and related substances assay methods. The manufacturing batch records may be examined for deviations from the norm. The disposition of the batch can

Table 7
Repeatability for NIR conformance testing of intermediate 1

Day	Analyst	Sample identification	NIR conformance	CI	Sample identification	NIR conformance	CI	Sample identification	NIR conformance	CI	Sample identification	NIR conformance	CI
1	A	II-B	Pass	4.00	II-G	Pass	4.68	II-C	Pass	4.88	II-D	Fail	5.77
1	A	II-B	Pass	3.48	II-G	Pass	3.68	II-C	Pass	4.57	II-D	Fail	5.27
1	A	II-B	Pass	3.27	—	—	—	—	—	—	II-D	Fail	6.28
2	A	II-B	Pass	3.23	II-G	Pass	3.97	II-C	Pass	4.53	—	—	—
2	A	II-B	Fail	5.00	II-G	Pass	3.10	II-C	Pass	4.25	II-D	Fail	6.44
2	A	—	—	—	II-G	Pass	4.65	II-C	Pass	3.42	II-D	Fail	7.55
3	B	II-B	Pass	3.85	II-G	Pass	3.92	II-C	Pass	3.98	II-D	Pass	4.75
3	B	II-B	Pass	3.27	II-G	Pass	3.79	II-C	Pass	4.90	II-D	Fail	5.85
3	B	—	—	—	II-G	Pass	3.56	II-C	Pass	3.54	—	—	—
4	B	II-B	Pass	3.16	II-G	Pass	3.83	II-C	Pass	3.96	II-D	Fail	5.99
4	B	II-B	Pass	3.40	II-G	Pass	4.47	II-C	Pass	4.04	II-D	Fail	5.28
4	B	II-B	Pass	3.36	—	—	—	—	—	—	II-D	Fail	6.59
Average CI				3.60			3.97			4.21			5.98
RSD				15.6%			12.7%			12.2%			13.3%

be decided based upon comparison of all these data to the appropriate specifications.

During the validation of the conformance test methods for the intermediates, no false-positives were observed for batches with low potencies near the specification limits. The sensitivity of the NIR conformance test for the intermediates was observed to be approximately 2–3% difference in potency. Samples from batches with potency differences less than this will sometimes pass and sometimes fail the conformance test. This sensitivity is of the same order as an HPLC assay method. The relative standard deviation for a single replicate of a typical HPLC potency assay for a pharmaceutical intermediate in our laboratory is approximately 1%. Owing to this random variation alone, about 5% of the time a batch potency assay result will be outside the range of 2% higher or lower than its true value. This variability sometimes results in false-negatives (and potentially false-positives) by HPLC. As a quantitative test, however, the variability of an HPLC assay may be reduced by replication. As a pass/fail test, the precision of an NIR conformance method cannot be improved by replication.

4. Conclusions

An approach was developed to create and validate analytical methods to perform NIR conformance testing on two pharmaceutical intermediates used in the manufacture of loracarbef monohydrate bulk drug substance using the wavelength distance method. Method calibration sets were developed from second-derivative NIR spectra for 30 representative batches of each intermediate. These calibration sets were used to test second-derivative NIR spectra for samples from newly manufactured batches by NIR conformance testing. An approach was developed to validate the methods for accuracy, selectivity, ruggedness and repeatablity. With this approach, samples from authentic intermediate batches, lower in potency than typical, were used to challenge the NIR conformance test methods.
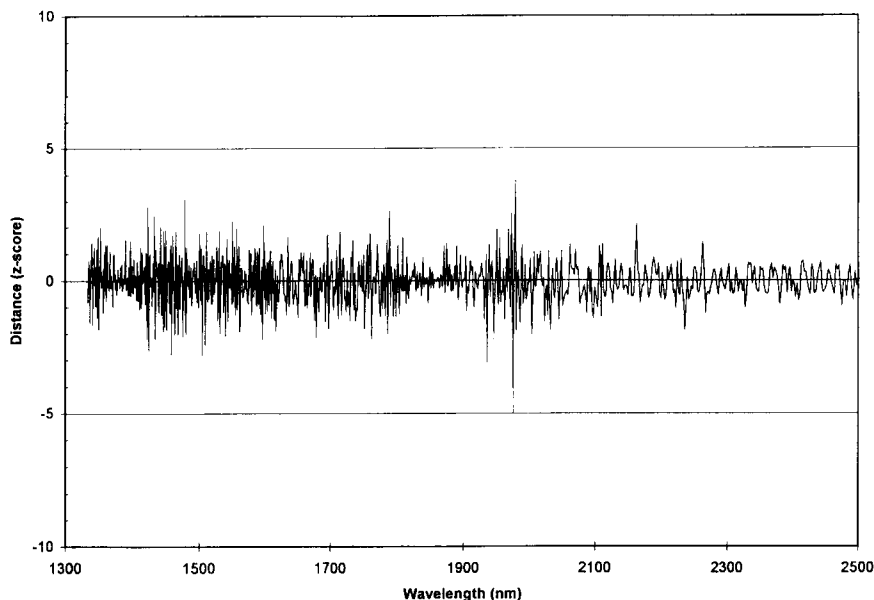
Fig. 5. Failing NIR conformance test for preparation of intermediate 1 Batch 11-B.

Authentic process samples were preferred owing to the likelihood of introducing differences merely by laboratory handling. The method validation demonstrated excellent selectivity of the NIR conformance test methodology for assurance of batch identity. As an additional advantage, the identification decision is automated versus a subjective comparison typical of some identity tests. The methods were found to be sufficiently rugged to fail consistently low-potency lots, near specification limits, and to pass consistently batches of typical potencies.

The methods were found to be sufficiently accurate and sensitive to discriminate samples with potencies 2–3% lower than typical. The sensitivity found in this study was larger than expected based on the references cited herein. The low-potency intermediate batches used in this study usually differed from typical batches in the relative amounts of related substances, which are normally present in all lots. One might expect the NIR conformance test method to be more sensitive to the presence of impurities not normally present in the calibration set.

This sensitivity would also be expected to depend on the degree of difference between the NIR spectrum for the intermediate and the impurity and on the strength and sharpness of the absorbances of the impurity. This reasoning demonstrates the importance of validation of the method for its intended usage.

Acknowledgements

Table 8
Stability of Sample Preparation for NIR Conformance on Intermediate 1

Sample identification	HPLC potency (%)	Description	NIR conformance	CI
11-B	98.9	Scan immediately	Fail	5.33
		10 min purge	Fail	5.36
		10 min on bench	Pass	3.58
11-G	99.3	Scan immediately	Pass	3.56
		10 min purge	Pass	3.88
		10 min on bench	Pass	4.35
11-D	94.9	Scan immediately	Fail	6.07
		10 min purge	Pass	4.63
		10 min on bench	Fail	5.26
11-E	93.5	Scan immediately	Fail	7.09
		10 min purge	Fail	8.12
		10 min on bench	Fail	6.90

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