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Near-infrared spectroscopy as an alternative to assess compliance of ampicillin trihydrate with compendial specifications

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Abstract: The suitability of near-infrared spectroscopy (NIRs) as an alternative to several compendial test methods such as identification, water content and assay, in the release procedure of drug substances is demonstrated with ampicillin trihydrate as an example. It is also shown that in cases in which a manufacturing process is well under control, a 'standard activity' can be assigned to a batch when its conformity with 'standard quality' is proven by means of its NIR spectrum. For that purpose a new quality parameter — the Conformity Index (CI) — is introduced.

Keywords: *Pharmaceutical analysis; quality control; near-infrared spectroscopy; validation; ampicillin trihydrate; Conformity Index.*

Introduction

Pharmaceutical analysts became interested in the applications of near-infrared spectroscopy (NIRs) in the quality control laboratory only after the introduction of the modern generation of monochromator instruments, in combination with high-speed computers and sophisticated software.

Although the highly overlapping absorptions in the NIR region (800–2500 nm) were once regarded as too complex for interpretation, today the NIR spectrum of a single substance or a multicomponent sample can be recorded and analysed in less than 1 min whereby a vast amount of information is obtained. Its simplicity and its precision combined with the speed with which the results are obtained, gives NIRs, in many cases, enormous advantages over traditional wet chemical methods, chromatography and other spectroscopic techniques.

For decades, NIRs has been used to determine water in agricultural samples and not surprisingly, water determinations in pharmaceutical powders and dosage forms by this technique have been reported [1–6]. Also, there is ample evidence that it is the ideal method of identification for large series of samples of incoming raw materials [4, 5, 7, 8]. The suitability of NIRs in the validation of a powder-mixing process has recently been described [9].

Other applications involve particle size determination, identification of polymorphism [4] and as a quantitative and qualitative detection method in thin-layer chromatography [10, 11]. Quantitative assays of active ingredients in the pure or in the dosage form, have been published by several authors [1, 3–5, 12–16].

In 1986, Whitfield [6] called NIRs 'an immature analytical method' but, with much foresight, he predicted a bright future in the pharmaceutical quality control laboratory. At the same time, however, NIRs was not expected to replace the more traditional techniques for potency assay, such as chromatography, in the final control procedure before product release. Both predictions have become true: NIR instruments are found in practically all the larger pharmaceutical QC laboratories. However, NIRs has been accepted by the FDA as the official assay method for only one veterinary product [6]. None of the referenced works [1–6] claimed NIRs to be a suitable method to ensure product strength, mainly because of the difficulty of obtaining a representative set of calibration samples. Most authors, however, see the enormous potential

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of the technique for raw material identification and as an in-process control method.

In this paper, it is demonstrated that NIRs is very suitable indeed to guarantee the identity of a substance like ampicillin trihydrate and to determine its water content. Further, it will be shown that the presence of a calibration curve is not always a prerequisite to ensure the compliance of a substance with the release specification for strength. The results of this study also justify the use of NIRs as an alternative to the compendial assay method.

Experimental

No reagents or reference substances are required because samples are analysed in the powder form 'as is', without any previous treatment and referenced against a spectrum derived from approved samples from the same source.

The equipment consists of a Perstorp Analytical NIR Scanning spectrophotometer model 5000 (range 1100–2500 nm) with an IBM Personal System/2 computer with co-processor, printer and adequate software (IQ^2).

Additional instrument specifications are: accuracy, ±0.5 nm; precision, ±0.015 nm SD; scan speed, 1.8 scans s^{-1}; data interval, 2.0 nm; photometric range, 3.5 Abs at 1100–2500 nm; linearity, 1% of reading; noise, ≤20 μAbs at 1100–2500 nm; stray light, <0.1% at 2300 nm.

Each spectrum recorded and analysed is the average calculated from 32 scans of that sample.

Results

Identification

To justify the implementation of NIR spectroscopy for the identification of ampicillin trihydrate, the following experiments were carried out:

The NIR spectra of 30 regular production batch samples, previously approved by applying the compendial methods, were recorded. The average spectrum was calculated and stored for use as the reference spectrum in the next experiment.

The NIR spectra of all chemically related substances circulating in this QC laboratory were also recorded. For each substance, a cosine value was calculated by an algorithm that treats the NIR spectrum or its first, second or higher order derivative as an N-dimensional vector, where N is the number of wavelengths of the spectrum. Comparison of the spectrum of the substance to be identified with the reference spectrum is accomplished by calculating the cosine of the angle between the vectors of both spectra. This cosine value is called the 'Spectral Match Value' (SMV) and may vary from −1 to +1, with +1 indicating the perfect match [5].

The relative standard deviation (RSD) of a single SMV measurement of an approved batch sample is as small as 0.025%, including the variation due to the differences between cells, and to filling and measurement.

The results (Table 1) demonstrate the suitability of NIRs to identify ampicillin trihydrate among such related substances as anhydrous ampicillin, ampicillin sodium, amoxicillin trihydrate and cephalexin monohydrate.

The spectra of about 500 production batches were recorded and the SMVs calculated. All samples were positively identified by comparing their IR spectra with the European Pharmacopoeia Chemical Reference Substance even if the SMV was found as low as 0.8679. Examination of the results showed that the SMVs of 16 batches with a batch history deviating from normal, were found to be below 0.9980 and ranged from 0.9978 to 0.8679. All 16 were found to be either mixtures of the trihydrate and the anhydrous form or found to be high in residual solvents and other impurities due to incomplete washing or drying of the batch.

On the basis of these results, it was decided to introduce NIRs for identification to replace mid-IR and to establish 0.9980 as the minimum value for a positive identity.

Table 1
Spectral Match Values (SMV) of related beta-lactam compounds

Compound	SMV
Ampicillin trihydrate	1.0000
Ampicillin anhydrous	0.2622
Ampicillin sodium	0.1204
Amoxicillin trihydrate	0.8881
Benzylpenicillin potassium	0.2557
Benzylpenicillin procaine	0.5028
Benzylpenicillin benzathine	0.2908
6-Aminopenicillanic acid	−0.0269
Cephalexin monohydrate	0.3176
7-Aminodesacetoxycephalosporanic acid	0.0095

Water content

Accuracy. To determine the water content of a sample by NIRs, a calibration curve is needed, covering the range of water contents to be expected. For established processes, the variation between batches is usually very small and therefore it will often be difficult to construct such a curve.

However, from time to time the ampicillin trihydrate process yields batches with water contents outside the compendial limits (12.0–15.0%). From these outliers, 10 samples with water contents ranging from 7.1 to 11.6% were selected. Together with samples found to be within the compendial limits, a suitable calibration curve was obtained, based on two wavelengths (1642 and 1930 nm). The other samples with deviating water contents were used to verify the validity of the curve thus obtained (see under 'Linearity and range').

The results found by the Karl Fischer (KF) titration, the official Ph.Eur.BP method, for 4952 approved production batches are approximately normally distributed around the average value of 13.1% with a standard deviation of 0.2%. The theoretical water content of this trihydrate is 13.4%. Once the calibration curve was obtained, water contents were also determined by NIRs in 474 of the 4952 batches. Both series of results are shown in Fig. 1 and their similarity is very close. The average value obtained with the NIR method is 13.2% with a standard deviation also of 0.2%.

Precision. The precision of the NIR water determination was established by the following experiment:

10 different cells were filled with powder from a well homogenized batch sample and measured;

— One cell was filled 10 times with the same powder and measured;

— One cell was filled once and measured 10 times.

The results in Table 2 show that the variation between cells is the largest source of variation. The relative standard deviation is 0.48% compared with 0.60% for the KF titration.

Linearity and range. The validity of the calibration curve is confirmed by the linearity of the plot of the KF versus NIR results for 17 batches (Fig. 2). The equation, calculated by the method of least squares, is

$$KF = -0.54 + 1.044\ NIRs,$$

Table 2
Precision of the water content determination by near-infrared spectroscopy (NIRs)

Total variation (10 different cells)	
Range	: 13.34–13.55%
Average	: 13.43%
s_{abs}	: 0.064%
s_{rel}	: 0.48%
Variation due to filling and measuring (1 cell)	
Range	: 13.59–13.72%
Average	: 13.64%
s_{abs}	: 0.041%
s_{rel}	: 0.30%
Variation due to measuring (10 times)	
Range	: 13.50–13.55%
Average	: 13.53%
s_{abs}	: 0.016%
s_{rel}	: 0.12%

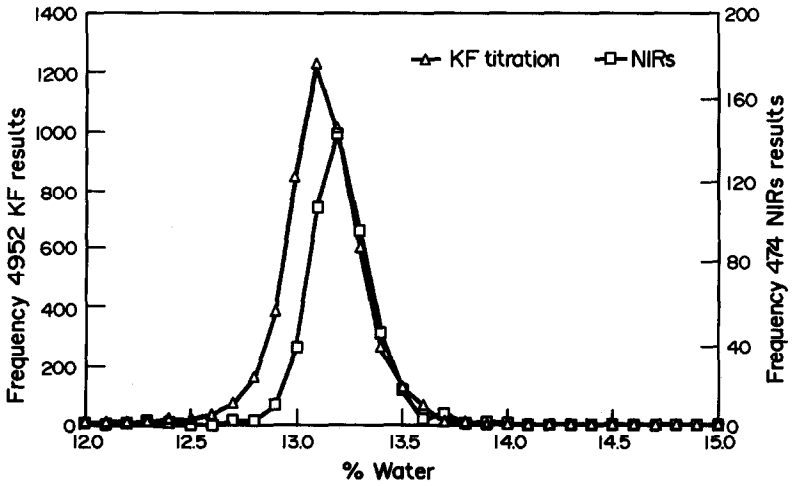

Figure 1
Water contents of ampicillin trihydrate found by Karl Fischer titration and NIR spectroscopy.

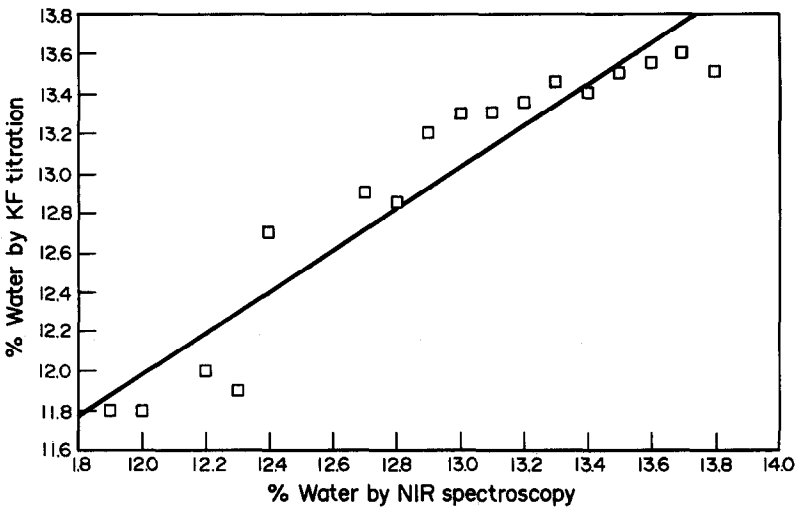

Figure 2
Correlation between water contents found by Karl Fischer titration and NIR spectroscopy in ampicillin trihydrate.

with $SD_{intercept} = 123$ and $SD_{slope} = 0.095$. The correlation coefficient is 0.946. The SDs indicate that the bias is not significantly different from zero whereas the slope is not significantly different from 1.

Ruggedness. This validity criterion is of limited importance because other instruments are not involved. However, if other instruments are involved in the testing of the same batches, a bias correction may be necessary. Normally, the slope should not differ between instruments.

It has been demonstrated in this laboratory that the results of the measurements are slightly dependent on the operator filling the cells. The best way to overcome this is to include this type of variation in the spectra selected for the reference spectrum.

Assay

When the manufacturing process of a drug substance is well established and well under control, the point may be reached that for certain parameters the variation between successive batches becomes of the same order of magnitude as the variation of the analytical method applied. In such situations, an actual result for a batch sample will not differ from the true value by more than the average value of the assay results obtained over a considerable period of time, provided these results are symmetrically distributed with reasonably small standard deviation.

Based on that philosophy, a system of assigning the average value (known as the 'standard activity') to each batch of ampicillin trihydrate for which the assay value was found to lie within a range of ±3 times the standard deviation of the assay method, was introduced several years ago. Such batches are declared to be of 'standard quality'.

In practice the current 'standard activity' of 85.5% is assigned to each batch of ampicillin trihydrate for which the assay result is found to be between 84.2 and 86.8%, being the 99.0% confidence limits of the 'standard activity' provided that no deviations from normal are reported in the batch manufacturing record. The justification for this practice is found in Fig. 3 in which the assay results of 4952 production batches of ampicillin trihydrate are collected together with 388 assay results found for the same control sample in a period of almost 2 years. This proof of 'conformity' of a batch of product with the 'standard quality' can however be done by NIR spectroscopy in a more precise, more sensitive and much quicker way.

Conformity

To establish the degree of conformity of a batch with the 'standard quality' the Conformity Index (CI) is introduced, which is calculated as follows.

For each of the 30 spectra recorded to obtain the reference spectrum (see under Identification) the second derivative is calculated. For each wavelength the average absorbance and its standard deviation is calculated. This results in an average or reference spectrum and a 'spectrum' of 666 standard deviations (due to

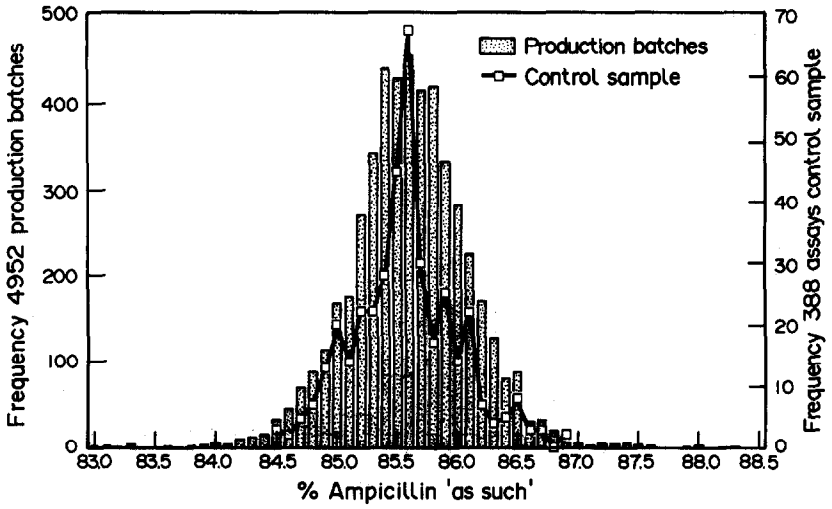

Figure 3
Distribution of ampicillin contents in production batches and in the control sample by the hydroxylamine colorimetric assay method.

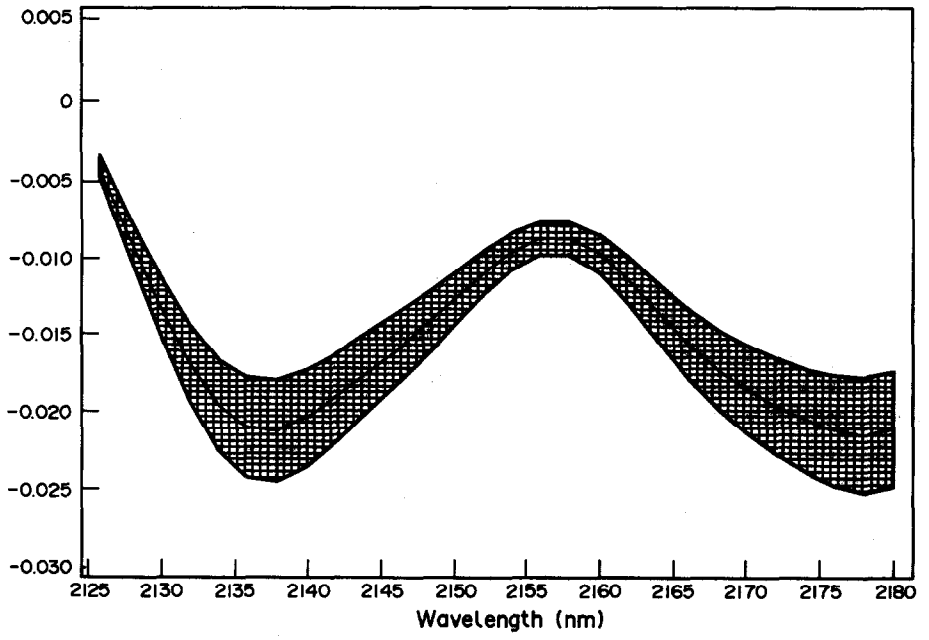

Figure 4
Part of the second derivative of the reference spectrum (2125–2180 nm) of ampicillin trihydrate with the ±5 SD limits.

the algorithm used, the information on 17 wavelengths at both ends of the spectrum gets lost). An example of a small section of the average spectrum and its ±5 SD limits is shown in Fig. 4.

The spectrum of the sample to be tested is recorded and the second derivative is calculated. For each wavelength from 1134 to 2466 nm, the value Q_w is calculated by dividing the absolute value of the difference between the absorbance at w nm of the sample spectrum and the reference spectrum by the standard deviation of the reference spectrum at the same wavelength. The CI is the maximum Q_w found for that sample.

For 324 samples of approved production batches the Conformity Indices have been calculated (Fig. 5). For the 17 batches with CIs higher than 5.0, a deviation from the normal process conditions had been reported but nevertheless they passed all traditional tests.

To verify the sensitivity of the CI for the

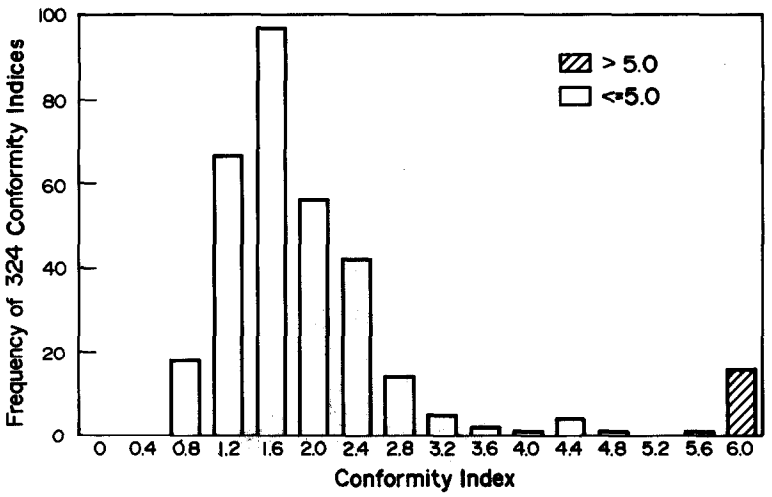

Figure 5
Distribution of the Conformity Indices found for 324 batches ampicillin trihydrate.

detection of deviations from normal processing, a number of samples of ampicillin trihydrate known to be different were collected and their CIs calculated. The results of this exercise are collected in Tables 3 and 4. In Table 3, examples are given of batches with an ampicillin content outside the range for the 'standard activity' due to the presence of small amounts of the anhydrous crystal form or a high level of residual water, together with several samples from competitors. It is obvious that these samples are easily distinguished from standard quality samples.

In Table 4 the effect of physical characteristics on both the SMV and the CI is demonstrated. Micronized or compacted material can easily be distinguised from untreated powder and the effect on both the SMV and the CI of blending 1% of magnesium stearate with ampicillin trihydrate and subsequent compacting is obvious. Recrystallization yields a product with a different impurity profile and a different particle size distribution leading to a significantly higher CI.

Discussion and Conclusions

Manufacturers of bulk pharmaceutical chemicals are entitled to establish the compendial compliance of their products with alternative methods, chosen for certain advantages, providing such alternatives lead to the same conclusions as the official methods. In case of doubt or dispute, methods and reference materials of the relevant pharmacopoeia are authoritative.

Table 3
Conformity Indices (CIs) of ampicillin trihydrate samples deviating from standard quality

Batch no.	Ampicillin content	CI
Content outside range for standard activity (84.2–86.8%)		
40643	87.2%	137
40979	83.1%	32
41226	84.1%	8
41255	83.1%	23
41424	88.2%	235
41443	86.9%	148
41492	87.9%	199
Products from competitors		
A		20
B		11
C		18
D		14

Table 4
Spectral Match Values (SMV) and Conformity Indices (CIs) of differently processed ampicillin trihydrate

Batch no.	SMV	CI
Micronized		
40900	0.9969	17
40901	0.9965	18
40902	0.9968	18
Compacted (no additives)		
95347	0.9981	14
95348	0.9976	16
95349	0.9932	25
Granulate (with 1% magnesium stearate)		
91250	0.9923	25
91251	0.9844	38
91252	0.9943	23
Recrystallized		
40549	0.9996	10
40550	0.9993	15
40551	0.9995	11

For well-established processes, it is possible that the QC laboratory is only confirming day-to-day variation of the analytical method itself. Having confirmed that this is the case with ampicillin trihydrate and several other products it was decided to investigate whether NIRs is suitable as a routine method to prove the conformity of batches produced daily, thus taking advantage of the obvious merits of this technique.

For the identification of beta-lactam compounds, the suitability of the Spectral Match Values to distinguish such related products from each other has been clearly demonstrated by the results collected in Table 1.

On the basis of the results presented, the conclusion is justified that NIRs is able to determine the water content of ampicillin trihydrate as accurately and as precisely as the traditional KF method.

It has also been proven that the newly introduced Conformity Index is able to detect small chemical or physical deviations. Therefore, this parameter not only allows the detection of such deviations, which are hardly noticeable by any other method, it is also a powerful tool for controlling manufacturing processes and for achieving constant quality.

The authors prefer as a criterion of compliance the use of the CI rather than the construction of a calibration curve based on a mixture of regular, treated and aged production samples and blends with sodium carbonate [3]. The use of samples from different sources reduces the sensitivity of the CI for deviations, due to the introduction of unnecessary variations.

All the results reported above justify the decision

(1) To implement NIRs as a routine method to replace in-house the official tests for identity and water content.

(2) To assign the standard activity to each batch for which the Conformity Index is found to be 5.0 or less.

(3) To approve and release for sale ampicillin trihydrate batches on the information obtained from the NIR spectrum between 1100 and 2500 nm instead of recording the mid-IR spectrum for identification and carrying out the KF titration and the hydroxylamine assay [17] on each batch. If a CI above the limit of 5.0 is found, an explanation and further testing by the official methods are required before the decision to release the batch or not can be taken.

(4) To carry out the official test methods for water and ampicillin content in only 1 out of every 10 batches. The results obtained (two a week) are assessed frequently to verify whether the value for the 'standard activity' mentioned on the product's label, remains justified.

A validation study proving the suitability of NIRs as an alternative method for ampicillin trihydrate has been presented to the FDA and had been well received.*

The purpose of this paper has been to inform those who are as yet unfamiliar with the enormous potential of this spectroscopic technique and its application. Further, it is also an attempt to convince those who are still sceptical about drawing conclusions based on results coming from a 'black box', of the fact that such conclusions are valid. In addition, the method is not only non-destructive, reagent-free, almost operator independent, often surprisingly sensitive, fast, precise and accurate, but can also give valuable additional information from the same spectrum obtainable by any other method.

It is hoped that the paper has demonstrated that NIRs is a very valuable analytical technique in the control laboratory of a pharmaceutical manufacturer. Applications of the technique are by no means limited to ampicillin trihydrate nor are the applications for ampicillin trihydrate limited to the ones described.

In subsequent papers from this laboratory other applications will be reported.

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*Note added in press: The FDA approved the use of NIRs and the deletion of the three official test methods.

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