Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 797–803, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00
Pergamon Press Ltd

The use of near infrared spectroscopy in the quality control laboratory of the pharmaceutical industry*

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Abstract: The suitability of NIR spectroscopy as an alternative to several compendial test methods is discussed. Using ampicillin trihydrate as an example it is demonstrated that eight quality criteria are controlled by recording the NIR spectrum of a batch sample and calculating its Conformity Index.

Keywords: *Near infrared (NIR) spectroscopy; pharmaceutical analysis; quality control; conformity index; C-plot.*

Introduction

For a long time the highly overlapping absorptions in the near infrared (NIR) region of 800–2500 nm were regarded as too complex for interpretation (Fig. 1). This is not surprising because these absorptions are overtones and combination bands of C—H, N—H and O—H vibrations observed in the mid-IR spectrum. These absorptions are not only far less intense but are also difficult to assign directly to functional groups of the sample as can be done with the fundamental bands in mid-IR. These disadvantages at first sight may however be of advantage in certain quantitative applications because: the absorptions are linear over a wide dynamic range and samples can be measured as such in reflectance or transmission without any preparation and without the use of solvents or any other diluent.

This makes NIR spectroscopy an attractive and in many cases, a superior technique compared with others as will be seen in this paper.

However, official pharmaceutical applications are still limited in number. The authors are aware of an assay entitled 'Piperazine in Drugs' published in the AOAC Official Methods of Analysis [1]. It is also known that the FDA has accepted NIR spectroscopy as the official method for determination of the lincomycin content in an agricultural premix of mainly soybean meal [2].

Pharmaceutical analysts only recently became interested in NIR spectroscopy after the introduction of the modern generation of grating instruments, like the Perstorp Analytical NIR scanning spectrophotometer model 5000 combined with a high-speed computer and sophisticated software.

The enormous amounts of samples of bulk antibiotic production in the authors' laboratory have to be analysed by tedious and often uninformative analyses. With most processes well under control analyses may also be tedious to carry out because there is normally little variation in the results. Therefore, a first objective was to see whether NIR spectroscopy could replace some of these compendial methods.

Applications

Identification

NIR spectroscopy is ideal for the rapid identification of samples from incoming raw materials. The total time required for filling the sample cell, the actual measurement and cleaning takes not more than 3–4 min.

The software supplied with the instrument enables the Spectral Match Value (SMV) to be calculated. The SMV is the cosine value between the sample spectrum and the reference spectrum, both regarded as a vector. A SMV of +1 means a perfect match between the two spectra [3].

The SMV of two classes of compounds have been determined in the authors' laboratory. For several very closely related cellulose deriv-

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.
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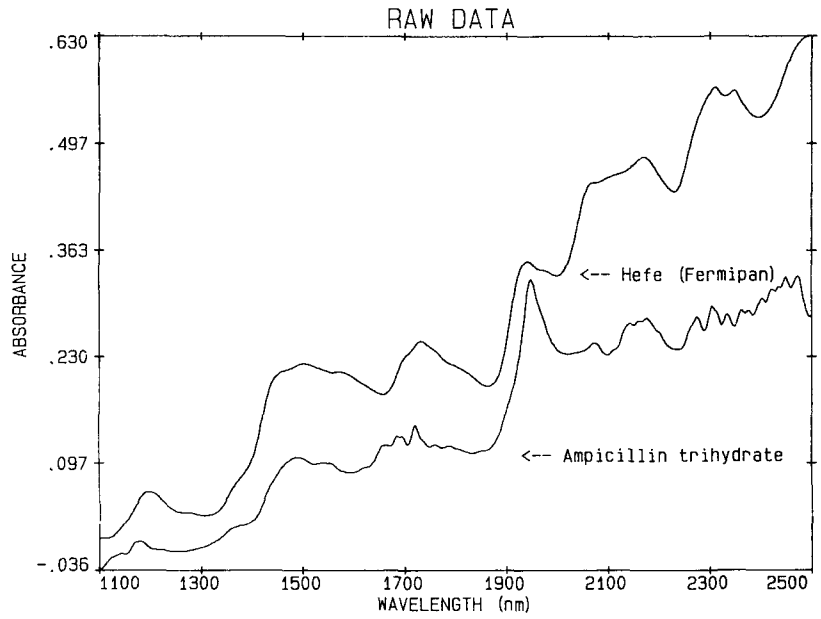

Figure 1
NIR spectra of dried bakers' yeast and ampicillin trihydrate.

Table 1
Spectral Match Values (SMV)* of nine cellulose derivatives matched against microcrystalline cellulose

Microcrystalline cellulose (MCC)	1.0000
Methylcellulose (MC)	0.7467
Methylhydroxyethylcellulose (MHEC)	0.7344
Carboxymethylcellulose Na (NaCMC)	0.7150
Methylhydroxypropylcellulose (MHPC)	0.7112
Hydroxyethylcellulose (HEC)	0.6954
Hydroxypropylcellulose (HPC)	0.5777
Methylhydroxypropylcellulose phthalate (MHPCP)	0.5487
Ethylcellulose (EC)	0.5066
Cellulose acetate phthalate (CAP)	0.3789

*From ref. 4.

atives, the SMV have been calculated with reference to microcrystalline cellulose [4]. The results are summarized in Table 1. The SMV for all the β-lactam compounds circulating in the QC laboratory are given in Table 2.

Since the fourth digit of the SMV is still significant, the conclusion is justified that the identity of these closely related products can be safely confirmed by NIR spectroscopy.

Water content

In pharmaceutical quality control water determinations are very common. For ampicillin trihydrate the water content is determined by the Karl Fischer titration. By chance the factory sometimes turns out samples with deviating water contents so it was possible to construct a calibration curve. To verify the accuracy of this spectroscopic method, 474 results obtained with this calibration curve have been compared with the results of the Karl Fischer titrations carried out over the years on 4952 batch samples. The agreement

Table 2
Spectral Match Values (SMV) of nine β-lactam compounds matched against ampicillin trihydrate

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

between the two methods is excellent whereas the precision of the NIR spectroscopic method is slightly better. Linearity and range are also good.

Ampicillin content

The routine assay method for ampicillin in the authors' laboratory is the hydroxylamine colorimetric method as described in the Code of Federal Regulations. When assay results of the same 4952 batches are compared with 388 results obtained with one and the same control sample, the conclusion is that the variation in ampicillin content in production batches is of the same order of magnitude as the variation in the assay itself. Every time a batch sample is assayed and the result is within the normal range, it is confirmed that the sample conforms to the usual or 'standard quality'. In such cases the 'standard potency' of 85.5% is assigned to the batch. Establishing this conformity can be done much easier by NIR spectroscopy.

Conformity

To this purpose the authors introduced a new quality parameter, the Conformity Index (CI). The CI is the largest value obtained by dividing the absolute difference in absorption between the sample spectrum and the reference spectrum (first or second derivative) for each datapoint by the standard deviation of the absorbance of the reference spectrum at that particular datapoint. For 324 normally tested and approved batches this CI was calculated and it was concluded that a batch with a CI of 5 or lower is of 'standard quality' in which case the 'standard potency' can be assigned (Fig. 2). For each of the 17 cases where the CI was found above 5, a deviation of the normal process had been reported. In such cases, the compendial methods will be applied before the decision can be taken to release or not the batch for sale.

Nonconformity may be due to chemical deviations such as a different impurity profile or a different water content or due to differences in the physical properties of the powder such as different particle size and distribution or the occurrence of amorphous or different crystal forms.

The sensitivity for physical differences is clearly demonstrated in Table 3. The authors are convinced that products with different crystal forms will have a CI far above 5 which makes the test on crystallinity, as required by the USP, superfluous when the sample is found to conform.

Thus, it is proposed that the compendial methods for identification, crystallinity, water content and ampicillin content could be replaced by NIR spectroscopy. The compendial methods should be carried out on only one out of every 10 batches for monitoring purposes.

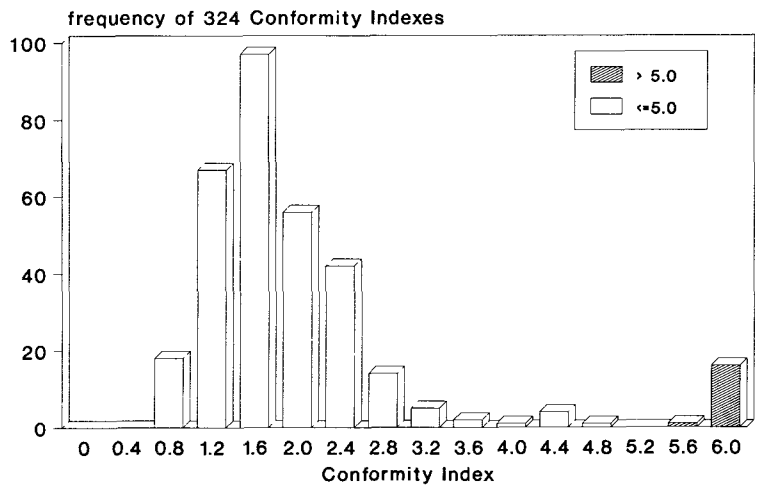

Figure 2
Distribution of Conformity Indexes of 324 approved batches of ampicillin trihydrate.

Table 3
Spectral Match Values (SMV) and Conformity Indexes (CI) of differently processed samples of ampicillin trihydrate

Batch no.	SMV	CI
Micronized		
40900	0.9969	17
40901	0.9965	18
40902	0.9968	18
Comparcted (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

Regulatory Aspects

All major pharmacopoeias allow manufacturers to use alternative methods for compliance testing. However, such alternatives have to be validated in order to demonstrate that interpretation of their results leads to the same conclusions. Once validated, such methods have to be submitted to the Registration Authorities of countries like the USA with strict regulations for drug substances.

The validation results have been submitted to the FDA and the authors had the opportunity to explain the method, its scientific merits and advantages during a presentation to the staff of the Antimicrobial Drugs Branch of the FDA. In the discussion it was made clear that the FDA has no objections against implementation of NIR spectroscopy as an in-house release test method if properly validated and reported in the annual update of the Antibiotic Drug Application. It can only be hoped that other Registration Authorities will follow the same attitude when it comes to the acceptance of non-compendial methods in the European Drug Master File.

Additional Applications

The application of NIR spectroscopy is not limited to ampicillin trihydrate; nor for this antibiotic is the application of this technique limited to the analytical criteria considered earlier in this paper.

The original software that was supplied with the instrument was of limited use in attempts to extract all the information present in the NIR spectrum. The IQ^2 program produces a parameter called Distance (renamed by the authors as the Conformity Index) as a simple figure, without even mentioning the wavelength at which this figure was found. The authors were, however, very interested in this wavelength because it might give an indication about the cause of the deviation.

Therefore, the software was extended to report not only this wavelength but also to produce a C-plot, that is a 'spectrum' of all the Distances calculated for that sample. For a normal batch of ampicillin trihydrate a typical C-plot is that in Fig. 3(a). However, sometimes the process is out of control and deviating plots are obtained [Figs 3(b) and 4]. These 'spectra' indicate immediately the cause of the deviation. Figure 3(b) shows a C-plot where there is an abnormal residual quantity of a reagent present in the product. Because its quantity in this sample has been assayed by GLC as 3200 ppm, the conclusion is that the CI of a sample will exceed 5 when the level of this reagent is above 1500 ppm. This is still below the rejection limit of 2000 ppm. The same is true for the two possible residual organic solvents when present in levels of about 1500–2000 ppm.

The C-plot in Fig. 4 shows the effect of the presence of anhydrous ampicillin in ampicillin trihydrate. The C-plot of anhydrous ampicillin against ampicillin trihydrate was recorded; a plot identical to Fig. 4 was obtained. With the CI measured (1100 at 2054 nm) and assuming linearity between the CI and concentration, it is possible to determine the amount of the anhydrous form of ampicillin in the trihydrate, on the basis of the CI found for a trihydrate sample at 2054 nm.

A nice proof of the linearity of the CI with concentration is demonstrated in Fig. 5 where the C-plots of mixtures of ampicillin trihydrate with 5 and 10% of benzylpenicillin are given.

Finally, another impurity can be controlled using the CI as a quality parameter. The starting material in the ampicillin synthesis is 6-APA; this is a β-lactam compound with an amino-group at the 6-position instead of the side-chain. To establish the sensitivity of the CI for the presence of residual 6-APA 5% of this compound was blended with ampicillin tri-

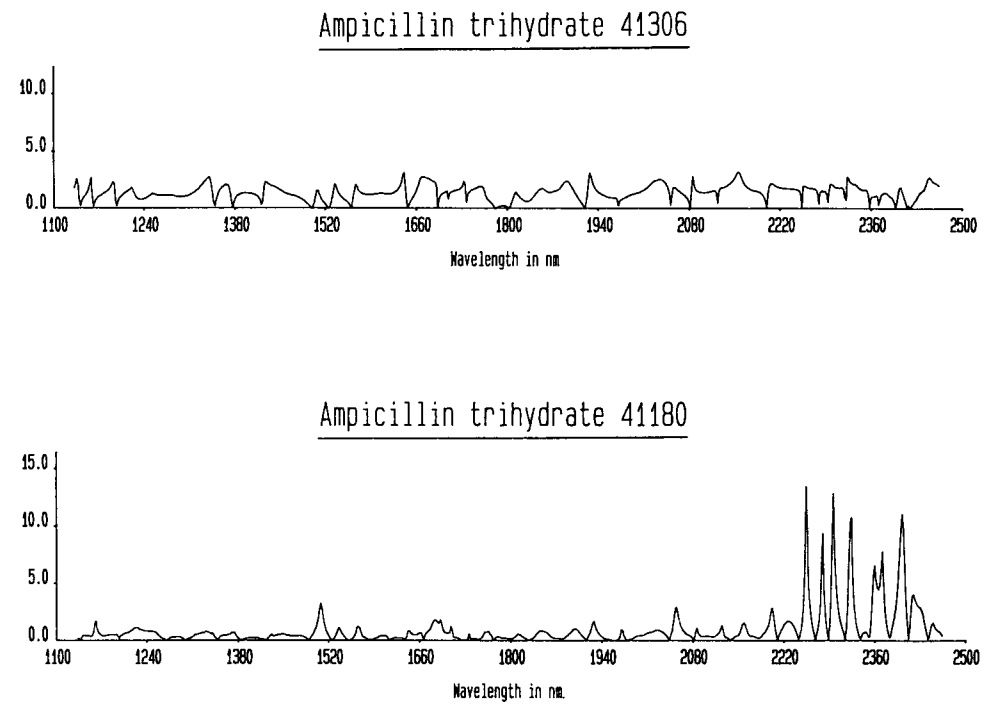

Figure 3
C-plots of standard quality ampicillin trihydrate (upper) and of a batch containing 3200 ppm of a residual reagent (lower).

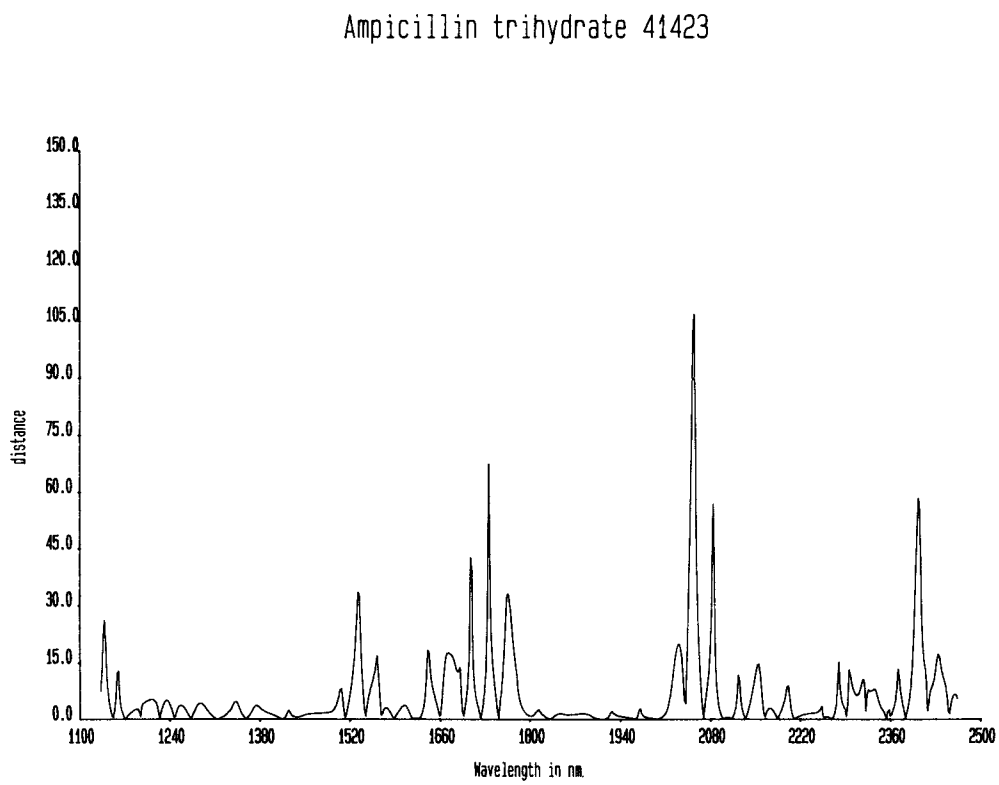

Figure 4
C-plot of ampicillin trihydrate with about 10% of anhydrous ampicillin.

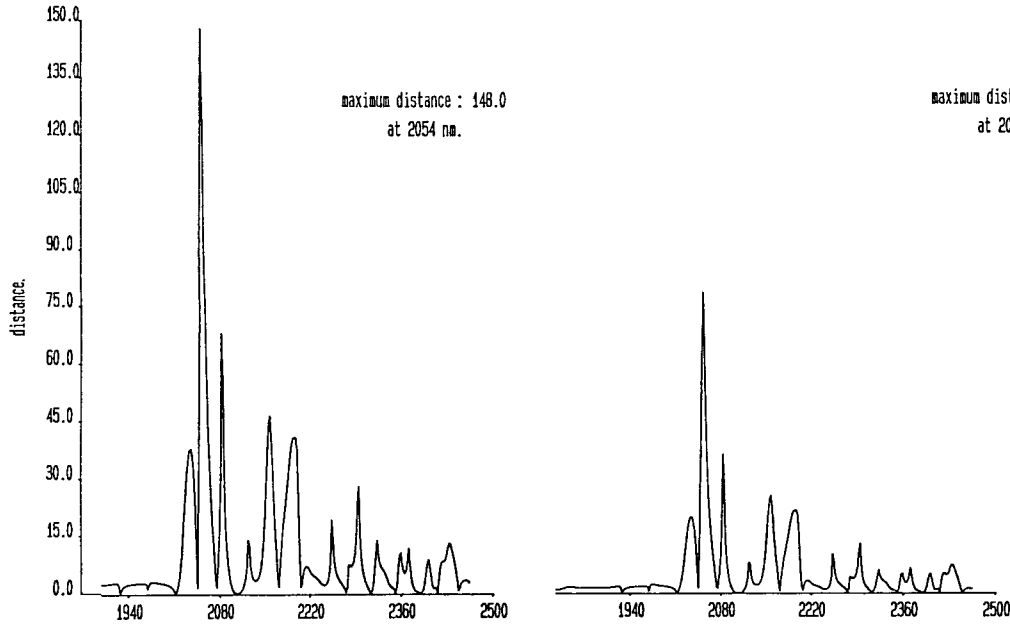

Figure 5
C-plots of ampicillin trihydrate blended with 10 and 5% of benzylpenicillin, respectively.

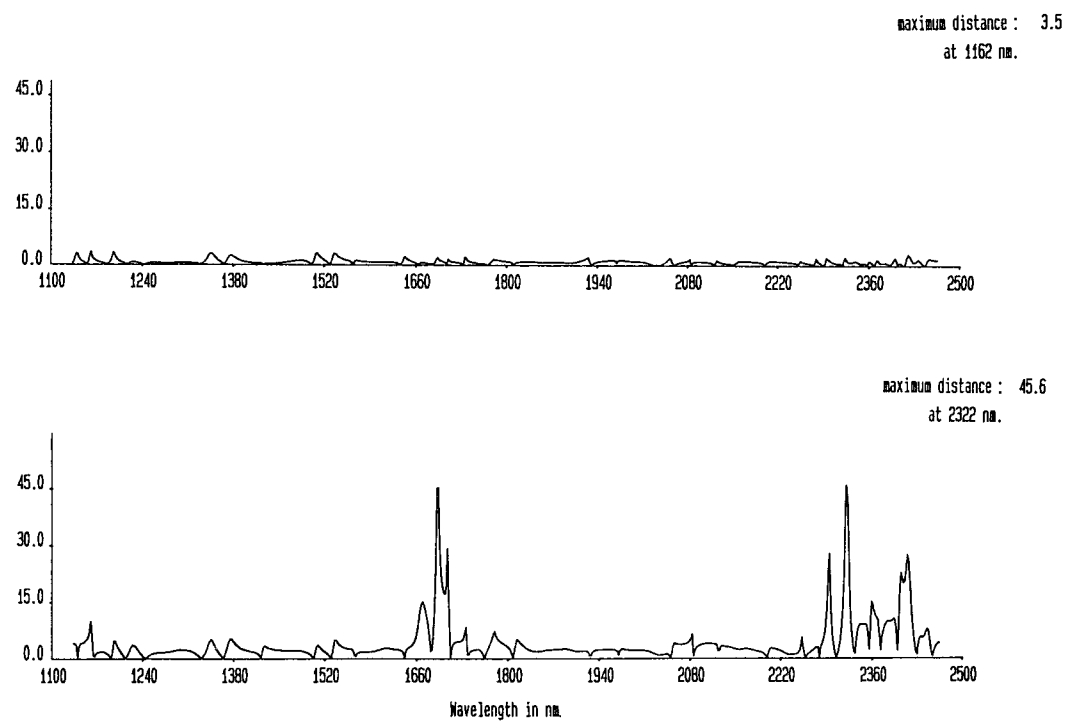

Figure 6
C-plots of ampicillin trihydrate and blended with 5% of 6-APA.

hydrate. From the CI plot (Fig. 6) it can be concluded that proportions of 6-APA above 0.5% will yield a CI above 5.

Conclusions

By recording an NIR spectrum of a sample of ampicillin trihydrate and calculating the Conformity Index, the following quality criteria are controlled: identity; crystallinity; water content; ampicillin content (total); ampicillin anhydrous fraction; residual reagent; residual organic solvents; and residual starting material.

Validation of the method demonstrated that several compendial methods can safely be replaced by NIR spectroscopy in the manufacturer's QC laboratory; the use of NIR spectroscopy gives better assurance of the quality of the drug substance than does application of the compendial methods alone. If the CI is found to be 5 or less, the batch is of 'standard quality' and 'standard potency' will be assigned.

NIR spectroscopy is a powerful tool for quality control laboratories in the pharmaceutical industry and may be applicable to the quality control of many raw materials.

Acknowledgements — This paper could only be presented thanks to the valuable assistance and contributions by Miss J.S. van der Does de Bye and Messrs M. van Andel, I.F. Matadin, M. Oosterbaan and J.A. van der Zalm.

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[*Received for review 15 May 1992*]