Journal of Pharmaceutical & Biomedical Analysis
Vol 7, No 3, pp 303–308, 1989
Printed in Great Britain

0731–7085/89 $3 00 + 0 00

Drug analysis by near-infra-red reflectance spectroscopy. Determination of the active ingredient and water content in antibiotic powders

S LONARDI,*‡ R VIVIANI,* L MOSCONI,* M BERNUZZI,* P CORTI,† E DREASSI,† C MURRATZU† and G CORBINI†

** Direzione Controllo Qualità, Settore Analisi Chimiche, Glaxo S p A , via Fleming 2, 37100 Verona, Italy*
† Dipartimento Farmaco Chimico Tecnologico, Università di Siena, via Banchi di Sotto 55, 53100 Siena, Italy

Abstract Near-infra-red reflectance spectroscopy is used to determine the active ingredient concentration and water content of an antibiotic powder (ceftazidime pentahydrate) The validity of predictive models for active ingredient concentrations and water content, constructed by means of the multiple linear regression technique is discussed A procedure is devised for the analysis of production samples, which, on account of the very limited range of concentrations, prove a difficult fit to the above-mentioned model The results obtained in testing formulation samples from 1 year's industrial production are used to illustrate the potential of the technique

Keywords *Near-infra-red reflectance spectroscopy, quantitative analysis, antibiotics, ceftazidime*

Introduction

Near-infra-red reflectance spectroscopy (NIRS) [1–3] was first introduced in the late sixties for routine determination of the major constituents of agricultural products [4–6] As the result of the availability of low-cost computers and software, the NIRS technique has made substantial headway over the past decade and has also come into use in the pharmaceutical sector [7, 8], in particular for qualitative analysis [9–14] The aim of the present study was to evaluate the possible applications of the NIRS technique for quantitative analysis, with particular reference to the determination of active ingredient concentration and water content of an antibiotic powder (the characteristics of which are summarized in Table 1) without implementing any form of isolation process

Apart from its technical and scientific aspects, such an investigation is also justified on practical and operational management grounds, in that the technique is simple and yields results rapidly

‡To whom correspondence should be addressed

Table 1
Characteristics of antibiotic powder analysed

Active ingredient	Ceftazidime
Theoretical active ingredient concentration	77 65%
Acceptability range for active ingredient	73 77 81 53%
Acceptability range for water content	11 5 15 0%
Excipient	Sodium carbonate

These characteristics, in conjunction with the reliability of the technique, enable analytical responses to be obtained in real time, with the additional possibility of performing a substantial number of replications The result is that the application of the technique can be used to full advantage if, rather than being confined to the traditional context of the chemico-physical laboratory, it is transferred to the production line Thus it affords continuous monitoring of the production process, thereby reducing costs and improving the quality assurance

Theoretical Background

In the NIR region (from 800 to 2500 nm) are to be found the absorptions relating to many significant chemical bonds including, C—H, N—H, O—H, S—H, C=C and C=O With the NIRS technique, the sample to be analysed is subjected to NIR radiation, part of the energy is absorbed by the surface layers of sample, whilst the rest is dispersed in all directions The light dispersed is related to the composition of the test sample, by means of an appropriate calibration the constituents of interest may be determined

The most widely used method of calibration is multiple linear regression (MLR), which uses a least-squares procedure to estimate the parameters of the linear calibration function The mathematical procedure for this can be found in many statistics textbooks [15, 16]

The regression equation normally used in NIRS is of the form

$$\% Y = Co + C_1 M_1 + C_2 M_2 + \quad + C_n M_n,$$

where $\% Y$ is the concentration of the chemical species under analysis, Co is the cut-off score for Y, and C_1, C_2, , C_n are the regression coefficients for the spectroscopic measurements M_1, M_2, , M_n obtained at the corresponding wavelengths λ_1, λ_2, , λ_n

Spectroscopic measurements are usually made on a set of samples of known composition ("manual values"), and the combination of wavelengths is sought which yields the best prediction of concentrations ("predicted values") In this search, a number of statistical parameters are used, such as, for instance, the standard error of calibration (SEC), the correlation coefficient of calibration (RC) and the F-ratio value (F)

Once the appropriate wavelengths have been found and the coefficients C_1, C_2, , C_n calculated, a new set of test samples is used to perform the calibration test The standard error of prediction (SEP) and the correlation coefficient of prediction (RP) are routinely used to test the validity of the calibration equation

Experimental

Apparatus

A Technicon Model No 450 RP NIR reflectance analyser was used, equipped with an IBM PCXT5 computer and Technicon APC-PICKS software package for quantitative determinations

Sample preparation

A set of production samples was selected from different batches suitably chosen in such a way as to represent the maximum variability of active ingredient and water content Over the past production year the concentration of active ingredient, as estimated by HPLC, ranged from 74 1 to 77 7%, whilst the water content, as measured by loss on drying over phosphorus pentoxide, ranged from 11 9 to 12 7%

To widen the spread of active ingredient concentrations and water content, two sets of laboratory samples were prepared, the first with a known concentration of active ingredient and the second with a known water content

To exact quantities of ceftazidime pentahydrate of known concentration were added exact quantities of anhydrous sodium carbonate, so as to yield a spread of active ingredient concentrations ranging from 70 6 to 82 9% To exact quantities of ceftazidime pentahydrate of known concentration were added exact quantities of anhydrous sodium bicarbonate and sodium bicarbonate decahydrate, so as to yield a spread of water content ranging from 11 1 to 14 9%

Both sets of samples were homogenized by mixing for 1 h on a vertical rotary mixer at 30 rpm

Determination of manual values

The concentration of active ingredient, in both laboratory and production samples, was determined by the following method Approximately 250 mg of pharmaceutical formulation, accurately weighed, were dissolved in 0 05 M ammonium phosphate and diluted to 250 ml with water Twenty microlitres of this solution were introduced into a liquid chromatograph, equipped with a Spherisorb ODS column (200 × 4 6 mm, i d , particle size, 10 μm) The mobile phase constituted 0 05 M ammonium phosphate/ acetonitrile (91 9, v/v), adjusted to pH 4 4 with an aqueous solution of 2% orthophosphoric acid The column temperature was maintained at 40°C and the mobile phase flow rate at 1 5 ml min^{-1}

The water content, in both laboratory and production samples, was determined by the following method Approximately 500 mg of powder, accurately weighed, were placed in a pre-set weighing bottle, left to dry for 48 h under vacuum over phosphorus pentoxide and reweighed

NIRS measurements

The NIRS measurements were carried out by introducing powder into polythene cells (5 mm film thickness) in amounts sufficient to fill them completely Samples were stratified, covered with microscope mounting slides and absorptions measured at the wavelengths listed in Tables 2 and 3

Results

In the first instance, an NIRS assay of active ingredient and water content in the

Table 2
Characteristics of regression equation constructed for determining active ingredient concentration

Regression parameters	Production Value	Production Wavelength (nm)	Laboratory Value	Laboratory Wavelength (nm)	Production + laboratory Value	Production + laboratory Wavelength (nm)
Co	79 3		122 6		109 6	
C_1	−651 0	2270	−9952 3	2336	−3144 7	1982
C_2	2201 0	2190	5821 1	2270	6622 9	1778
C_3	1264 0	1982	6385 5	2180	1842 6	1940
C_4	−2842 1	2100	−2514 8	1778	−4864 6	1722
C_5					−485 3	1445
SEC	0 83		1 45		1 03	
RC	0 650		0 973		0 967	
F	14 6		179 1		128 2	
SEP	2 09		1 89		1 36	
RP	0 421		0 962		0 951	

Table 3
Characteristics of regression equation constructed for determining water content

Regression parameters	Production Value	Production Wavelength (nm)	Laboratory Value	Laboratory Wavelength (nm)	Production + laboratory Value	Production + laboratory Wavelength (nm)
Co	14 2		13 6		33 7	
C_1	333 5	2336	−1109 0	2336	−1434 8	2348
C_2	−523 7	2270	1665 1	2190	1303 0	2270
C_3	155 7	2208	−601 0	1759	372 3	1982
C_4	33 9	1940			−306 7	1445
SEC	0 18		0 30		0 32	
RC	0 466		0 971		0 965	
F	5 9		211 5		160 2	
SEP	0 28		0 41		0 59	
RP	0 298		0 955		0 708	

pharmaceutical formulation of production samples was performed after ascertaining good instrumental and analytical reproducibility

The same sample measured 10 times consecutively yielded a relative standard deviation (RSD%) of 0 22, whilst the same analysis, repeated 10 times, yielded an RSD% of 0 39 After a brief investigation in all powder samples available, 29 were selected on the basis of their content and the difference between manual and predicted values With these samples, a regression equation was constructed for the active ingredient, and another for the water content The characteristics of these equations are summarized in the "Production" columns of Tables 2 and 3 Another 10 powders were used as unknowns to evaluate the SEP and RP (Tables 2 and 3)

The results obtained for the active ingredient show that the errors fall within the ±5% range, taken as the acceptability limit for the formulation analysed The RC and RP correlation coefficients, however, are low and the *F* value rather unsatisfactory

The results obtained for water content are unreliable SEC and SEP values are not excessively high, but the RC and RP correlation coefficients proved to be unacceptable, as was the *F* value

These initial results determined the subsequent phases of the investigation It was assumed that the poor quality of the regression equations was due to the excessively narrow concentration ranges Therefore it was decided to prepare powders with known active ingredient concentrations and water contents so as to have wider concentration/ content ranges The results obtained with laboratory samples alone are summarized in the "Laboratory" columns of Tables 2 and 3 These show good values for all statistical parameters with regard to both the active ingredient and water content

Using the regression equations obtained, a set of production samples was then tested The resulting data, when compared with those obtained using traditional techniques, again failed to be entirely acceptable

On the basis of this observation, new regression equations were constructed using mixed production and laboratory samples The best results, given in the "Production" and "Laboratory" columns in Tables 2 and 3, are those obtained with a production to laboratory sample ratio of 1 1 In both cases, the statistical parameter values are satisfactory and lead one to expect reliable assays even for powders of unknown content

Operating with these latter regression equations, samples representative of an entire year's production were tested, evaluating the distribution of the percentage differences between values obtained using NIRS and those obtained using traditional techniques (residuals %) Figure 1 plots the frequency distribution of residuals % obtained between NIRS and HPLC values for active ingredient, and between NIRS and loss on drying values for water content

Discussion

Operating on production samples means making allowance in the calibration for all the variables arising from both production technologies and from the chemico-physical characteristics of the raw materials used But the concentrations generally fall within very limited ranges, which tend to be much narrower than the acceptability limits for the formulation

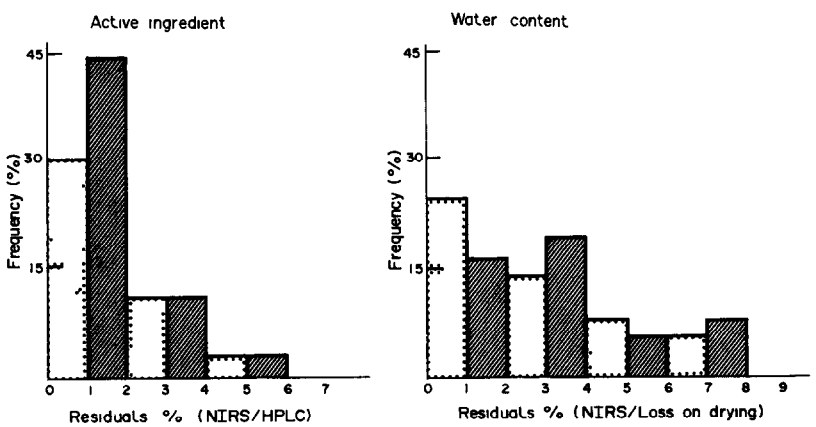

Figure 1
Distribution of percentage differences between values obtained using NIRS and those obtained using traditional methods in the analysis of samples representative of 1 year's production

This means that it is necessary to prepare laboratory samples so as to be able to broaden the calibration intervals Laboratory samples, however, do not possess all the characteristics arising from the technological process, with the result that the regression equations constructed using laboratory samples alone are found to be useless for the analysis of production samples

In fact it was established that, for the purposes of regression analysis, a total of 20 powders is a sufficient number if one is using an overall concentration range within ±10% of the theoretical value Satisfactory results were obtained with a laboratory/ production sample ratio of 1 1 The problems relating to the way the sample is to be presented definitely appear to have been solved, as demonstrated by the RSD% value obtained on exposing the same sample several times, consecutively, to spectroscopic measurement

When testing samples representative of an entire year's production, it was observed that differences in batches of raw materials used in the manufacture of the pharmaceutical formulation have no significant effect upon the analytical results The residual percentages are mostly distributed in the 0–3% range for the active ingredient and in the 0–5% range for the water content

The NIRS analysis technique used in the present study thus is proved to be reliable for the assay of the solid-state system This characteristic, together with the simplicity of the operations involved, the rapidity with which instrumental responses are obtained (approx 30 s) and the simultaneous determination of the constituents lead to the conclusion that the NIRS system is particularly suitable for use in production control operations

References

[1] D Wetzel, *Analyt Chem* **55**, 1165A–1176A (1983)
[2] M Bernardini, E Fedeli and D Baroni, *Riv Ital Sostanze Grasse* **62**, 357–364 (1985)
[3] B Buchanan and D Honigs, *Trends Analyt Chem* **5**, 154–157 (1986)
[4] J Hart, K Norris and C Golumbic, *Cereal Chem* **39**, 94 (1962)
[5] K Norris, *Agric Engng* **45**, 370 (1964)
[6] C Watson, *Analyt Chem* **49**, 835A–840A (1977)
[7] E Ciurczac and T Maldacker, *Spectroscopy* **1**, 36–39 (1986)
[8] E Ciurczac, R Torlini and M Denmkowicz, *Spectroscopy* **1**, 36–39 (1986)
[9] D Honigs, J Freelin, G Hieftje and T Hirschfeld, *Appl Spectrosc* **37**, 491–497 (1983)
[10] I Cowe and J McNicol, *Appl Spectrosc* **39**, 257–266 (1985)
[11] D A Tunnel, *Analyt Proc* **23**, 299–300 (1986)
[12] H Mark, *Analyt Chem* **59**, 790–795 (1987)
[13] H Mark and D Tunnell, *Analyt Chem* **57**, 1449–1456 (1985)
[14] I Cowe, J McNicol and D Cutherberston, *Analyst (Lond)* **110**, 1227–1232 (1985)
[15] N Draper and H Smith, in *Applied Regression Analysis*, 2nd edn Wiley, New York (1981)
[16] G W Snedecor and W C Cochran, in *Statistical Methods* Iowa State Univ (1967)

[*Received for review 16 December 1987, revised manuscript received 3 October 1988*]