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Quantitative determination of polymorphic forms in a formulation matrix using the near infra-red reflectance analysis technique*

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Abstract: An analytical method based upon near infra-red reflectance measurements on solid samples is described for the determination of the polymorphic transformation of an active ingredient in a solid dosage form matrix. Calculations were performed using multiple linear regression techniques. The method was applied to mixtures of two polymorphic forms of a new chemical entity in a formulation matrix and gave recoveries close to the expected values with an acceptable reproducibility.

Keywords: *Polymorphism in pharmaceutical preparations; multiple linear regression techniques; near infra-red reflectance analysis.*

Introduction

The polymorphism of active ingredients is an important phenomenon to be considered in pharmaceutical research since it may significantly affect the bioavailability of drugs. This solid state property is frequently observed in drug substances [1–4]. According to Burger [5], 36% of the active ingredients mentioned in the European Pharmacopoeia may be polymorphic; the proportion is 64% if pseudopolymorphism is included. It is essential to detect polymorphism in selecting the most appropriate crystalline form of a drug in relation to its bioavailability; it is also important to be able to assay polymorphic transformations to ensure physical stability of the selected crystal during manufacturing processes such as drying, grinding, compression and during stability studies on solid dosage forms.

Most of the published methods are concerned with the detection and characterization of the different crystalline forms of pure active ingredients and use differential scanning calorimetry (DSC), infrared (IR) and X-ray diffraction techniques [6–14]. Reviews of the available analytical methods have been presented [1, 15–17]. Some have been used for quantitative determination of the different polymorphic forms of a compound when mixed together; examples are differential enthalpic analysis for pentobarbitone [18] and IR spectroscopy for chloramphenicol palmitate [19] or for other organic compounds [20].

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Some workers have examined the polymorphic transformations which may occur during the manufacturing process for pharmaceutical preparations. They used IR spectroscopy, DSC, thermogravimetry, thermomicroscopy or X-ray diffraction to detect qualitatively solid state transition [21–25]. Very few investigations have been dedicated to the quantitative analysis of polymorphic forms in a pharmaceutical dosage form; IR spectroscopic and X-ray diffraction methods have been used for the assay of chloramphenicol palmitate, form A, in commercially available suspensions [26, 27].

Most polymorphic forms exhibit differences when examined by mid-IR spectroscopy. Near-IR spectra are related to mid-IR spectra; near-IR absorbances are overtones and combinations of mid-IR absorbances. Furthermore, near-IR analysis allows the assay of components with linear response in the solid state by diffuse reflectance measurements. For these reasons the near-IR reflectance analysis technique (NIRA), developed by Norris [28] and recently discussed by Wetzel [29] appeared to be the appropriate analytical method to quantify the mixed polymorphic forms of an active ingredient when present in a solid-state formulation.

The present study is concerned with the quantitative determination of polymorphic forms present in a formulation matrix using the NIRA technique on a new chemical entity with analgesic properties, SC-25469. This new chemical entity may exhibit two polymorphic forms, α and β. The β-form was selected for use in solid dosage forms because of its greater bioavailability. Although the β-form is the more stable, it may be transformed to the α-form under pressure by enantiotropy. It was for this reason that the β-form of SC-25469 had to be quantified during the development of solid dosage forms.

Experimental

Chemicals

The active ingredient used in this study may exhibit two polymorphic forms β and α, which were obtained as follows:

Form β was recrystallized from toluene–cyclohexane (2:1, v/v); fine needles were micronized (2–50 μm) as this form was intended to be used in formulation developmental work; m.p. 146–147°C.

Form α was recrystallized from toluene; rectangular plates (mostly 60–140 × 190–230 μm) were used without micronization as this form was not stable during such a process; m.p. 136–137°C.

A simulated matrix of a solid dosage form was prepared by dry-mixing of the following excipients:

	% (m/m)
Anhydrous dibasic calcium phosphate	80
Polyvinylpyrrolidone (Kollidon K30)	10
Magnesium stearate	1
Polyvinylpolypyrrolidone	9.

General procedure

Seven 1-g standard mixtures containing 50–35% of form β and 0–15% of form α in the formulation matrix were prepared by dry-mixing of the weighed quantities of each component in order to simulate the polymorphic transformation τ of form β to form α in a solid dosage form (Table 1).

Table 1
Standard mixtures

Mixture	Composition form β	(% m/m)* form α	matrix	Transformation (%)
1	50.0	0	50.0	0
2	47.6	2.5	50.0	4.9
3	44.9	5.0	50.1	9.9
4	42.5	7.6	50.0	15.2
5	40.0	10.0	50.1	20.0
6	37.4	12.6	50.0	25.2
7	35.0	14.9	50.1	29.9

* Rounded to the first decimal figure.

Each standard mixture was analysed in triplicate by reflectance spectroscopy. The diffuse reflectance data R, corresponding to the transmittance of transmission spectroscopy, were collected at 19 wavelengths; the data were then transformed in the absorbance mode, $\log_{10}(1/R)$, in order to establish linear relationships between reflectance data and the content of form β, form α, and the corresponding polymorphic transformation τ in the formulation according to the following model:

$$\%C = F_0 + F_1 \log 1/R_1 + F_2 \log 1/R_2 +,\ldots, + F_{19} \log 1/R_{19}, \quad (1)$$

where %C is the concentration of component C in the mixture (% m/m); F_0 is the constant calibration value depending on the analytical apparatus; $F_1,F_2,\ldots,F_{19}$ = specific calibration coefficients for the component C at wavelengths 1,2,...,19; $R_1,R_2,\ldots,R_{19}$ = diffuse reflectance measured at the wavelength 1,2,...,19.

For each component to be assayed in the formulation, the most characteristic wavelength, that is the wavelength for which the reflectance data in the absorbance mode are best correlated with the exact contents of the standard mixtures, was selected by linear regression analysis.

Similar multiple linear regression calculations for 2,3,4,..., wavelengths were performed according to equation (1) until the multiple correlation coefficient $r2$ was acceptable. Among the regression models for 2,3,...,n wavelengths, the calibration model was selected as the one which presented the highest value for the multiple correlation coefficient and the best values for the usual statistical regression parameters.

The multiple linear calibration models selected for assessing the β- and α-components as well as determining τ in the formulation matrix were then applied on three spiked samples in order to check the accuracy and the precision of the method. Spiked samples were prepared by weighing exact quantities of each component in a known amount of the formulation matrix; the samples were then dry-mixed by means of a mechanical shaker for 5 min.

Equipment

Near-IR diffuse reflectance measurements were performed on an InfraAlyzer 400 (Technicon) apparatus with 19 wavelength-filters ranging from 1440 to 2350 nm, using the integrating sphere with 2 PbS detectors and the solid sample cell UK1 of which the sample depth is about 100 μm.

The multiple linear regressions were calculated with a Hewlett–Packard microprocessor (HP-85) using statistical software designed by Technicon for quantitative applications.

Results and Discussion

Preliminary NIRA spectra

Qualitative spectra were recorded between 1100 and 2500 nm for each pure β- and α-crystalline form (Fig. 1a) and for the standard mixtures 1, 4 and 7 (Fig. 1b) using an InfraAlyzer 500 (Technicon). Pure chemicals exhibited sufficient differences in terms of absorbance wavelengths and absorbance values to suggest that quantitative assessments were possible. Spectra of standard mixtures confirmed that quantitative determinations were possible even if the qualitative differences were not apparent when these chemicals were mixed in the formulation matrix.

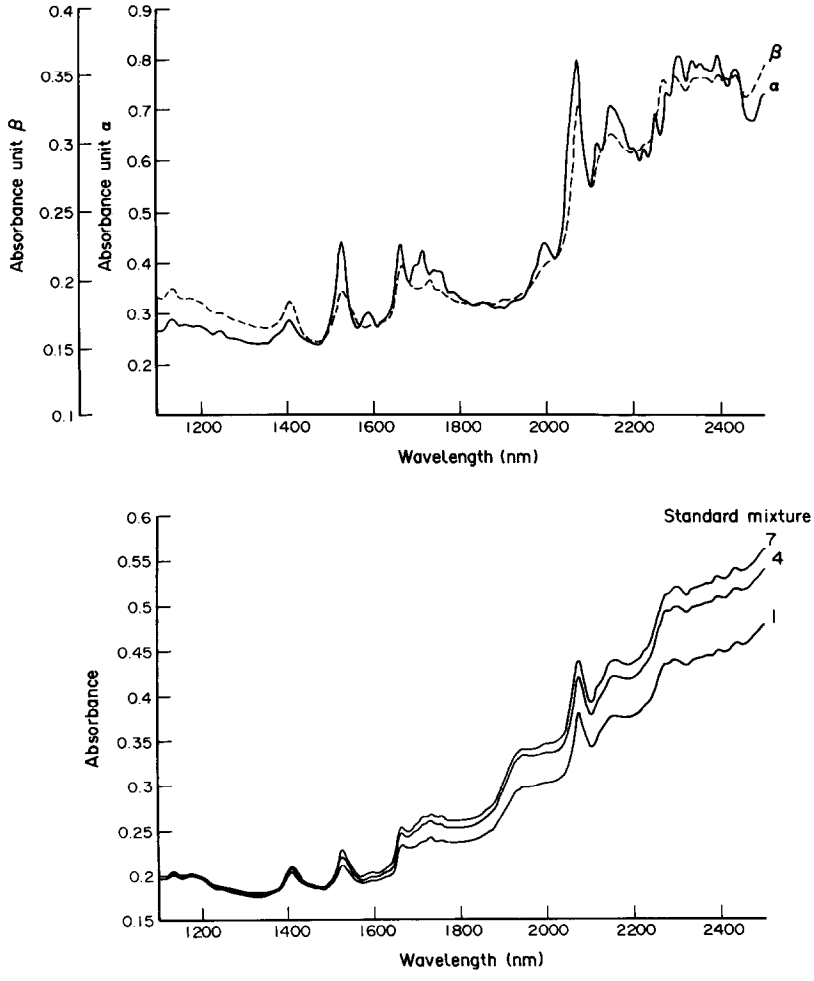

Figure 1
NIRA spectra. (a) pure α- and β-forms; (b) standard mixtures 1, 4 and 7.

Calibration models

For each component to be quantified in the matrix, multiple linear regressions (according to equation (1)) with 2–6 wavelengths were calculated for all the possible combinations of 19 wavelengths. Among these 5 calibration models, the 5-wavelength model was selected as it presented an optimum value of the multiple correlation coefficient $r2$ for every component (middle of the asymptotic part of the curve represented in Fig. 2).

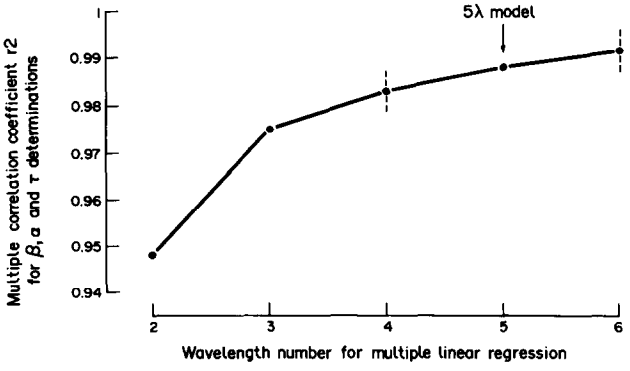

Figure 2
Choice of the multiple linear regression model.

The wavelengths selected for measurements and the 5-wavelength calibration models suitable to evaluate the percentage of β, α and τ in the formulation matrix were established as follows:

$\%\beta = 27 + 4722A_{11} - 12630A_{14} + 228A_{15} + 6329A_{17} + 1422A_{19}$,

$\%\alpha = 22 - 4694A_{11} - 337A_{13} + 14083A_{14} - 8553A_{17} - 505A_{18}$,

$\%\tau = 48 - 9461A_{11} + 25186A_{14} - 443A_{15} - 12604A_{17} - 2826A_{19}$,

$A_{11}, A_{13}, \ldots$: log $1/R$ for the wavelength–filter numbers selected. The filter numbers 11, 13, 14, 15, 17, 18 and 19, corresponded to wavelengths of 1818, 2100, 1759, 1940, 1722, 1445 and 1680 nm.

Results for spiked samples

The 5-wavelength models were used, with diffuse reflectance data collected at the selected wavelengths on the 3 spiked samples, to determine the percentages of β- and α-forms and the corresponding polymorphic transformation τ in the formulation matrix. The results, individual and mean values are reported in Table 2.

In comparison with the theoretical amounts, the predicted values were found to represent nearly 100% recovery and were determined with good precision, the relative standard deviation of the mean ranging from 0.1 to 0.9%.

The predicted values were also calculated with the 3-, 4- and 6-wavelength-models for polymorphic transformation τ. The comparison of the mean values using the 4 different models confirmed that the 5-wavelength model was the most appropriate to assess the polymorphic transformation of the active ingredient of interest in the formulation matrix with the best reproducibility.

Table 2
Results* for spiked samples

	Form β			Form α			Transformation		
	found	predicted values		found	predicted values			predicted values	
Sample	weight	individual	mean	weight	individual	mean	theoretical	individual	mean
1	49.9	49.7 49.9 50.2	49.9	0	2.1 −0.6 −0.8	0.2	0	0.6 0.2 −0.4	0.1
2	47.0	47.4 46.6 46.6	46.9	3.2	3.4 3.4 3.2	3.3	6.3	5.1 6.7 6.8	6.2
3	36.5	37.0 36.2	36.6	13.5	13.4 13.6	13.5	27.1	25.9 27.4	26.7

*Results are expressed as % m/m.

Conclusion

The near-IR reflectance analysis technique appears to be a suitable method to follow the polymorphic transformation of active ingredients in solid dosage forms and could be applied to other pharmaceutical products such as ointments. The main advantage of this technique is that it permits large quantities of material (1 g or more) to be analysed, thereby eliminating sampling error and sampling-induced heterogeneity.

In practice, it is a simple and fast method which only requires the use of a microprocessor of sufficient capacity to perform the needed multiple linear regression calculations and the statistical comparisons of all these possible regressions.

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