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X-RAY QUANTITATIVE DETERMINATION OF POLYMORPHISM IN PHARMACEUTICALS

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Abstract X-Ray diffraction may be applied successfully for quantifying polymorphism in raw materials and dosage forms. Examples of determinations and studies of transformation on aging are given.

INTRODUCTION

The correlation between solid state behaviour of drug substances and bioavailability of the dosage form is well known (1-4). Furthermore all properties of solid state like stability and processability are characteristics of each form. Transformations during processing and storage have been observed. For pharmaceutical development, quantitative determination of polymorphs is necessary.

Different methods are generally used: IR, NMR, DSC, X-ray diffraction (XRD).Every crystal form of a compound produces its own characteristic X-ray diffraction pattern, that allows identification and quantification of raw materials and dosage forms.

Our objective was to develop methods of quantification of mixtures of polymorphs in drug substance and in dosage forms, then to study raw materials and dosage forms on aging.

INSTRUMENTATION

X-ray diffraction patterns were obtained on a Philips compact X-ray Diffraction Analyser system 1840 using copper radiation (40 kV, 30 mA) with a Nickel filter (Cu Kα = 1.5418 Å).

Infrared spectra were recorded using a Perkin-Elmer 398 spectrometer. All samples were prepared as nujol mulls.

Differential Scanning Calorimetry, Perkin-Elmer instrument, DSC-2 or DSC-7 was used.

RESULTS

Optimization

Prerequisite for quantitative determination are as follows: a known number of phases; available standards; artefacts are avoided; content uniformity of the mixtures of standards used is good; peak heights should be reliable indicators of concentration.

For accuracy and good detection limits, the resolution of peaks, sensitivity of the instrument signal to concentration and the sample preparation are factors to be optimized.

In X-ray Diffraction the particle size, the amorphicity, the absorption effects, and preferred orientation are factors which have to be experimentally studied, otherwise the peak intensity is not proportionnal to concentration (5-9).

For determination the peak area or peak height, the use of an internal standard and sophisticated mathematical treatment have been proposed (10). Some quantitative methods have been published in the pharmaceutical area (11-15).

The identification of raw material is possible by means of d values of the interplanar spacings and the relative intensities as, for example, the case of Edetic acid in NF XVI. We could identify successfully Cephalexin as described in literature, only after correct grinding.

For optimizing quantitative XRD determination of polymorphs, we first isolate and manufacture standards, analysing them by means of IR, DSC, TG and XRD in order to confirm their suitability. We try to obtain them milled with the same procedure.

The mixtures are ground and analysed at least 5-6 times. Slow scan rates 0.001 or 0.002 2θ/s. are used in order to optimize accuracy. The peak height is calculated using the optimum scale of the recorder. Variability of each suitable peak is calculated and the mean value used in the calculation of linearity. Figure 1 and table I deal with a typical example for a new drug substance. 3 peaks were selected from first calibration curves using between 10 and 50% form A in B. New

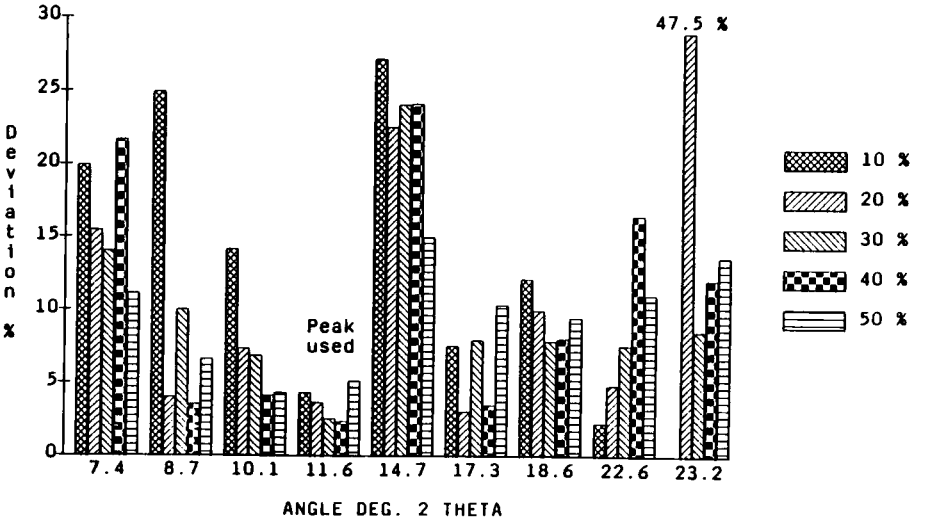

FIGURE 1 Peak height deviations for different concentrations of A

TABLE I Standard deviations of mixtures of form A in B (s)and linearity at 3 different angles

Angle 2θ	2%	4%	6%	8%	10%	correlation	slope	interc.
7.4°	s:24%	s:40%	s:17%	s:27%	s:29%	0.993	31	-9
11.6°	s: 5%	s: 5%	s: 3%	s: 5%	s: 6%	0.999	19	+98
17.3°	n.d.	n.d.	s: 7%	s: 7%	s: 7%	0.984	18	+57

calibrations were recorded between 0 and 10 % A and usual batches analysed. The results demonstrated a limit of detection at about 5 % A for the peak 2θ:17.3° due to insufficient resolution, excellent correlation for peak 2θ:11.6° and insufficient accuracy for peak 2θ:7.4° due to higher variability of measurements.

The peak 2θ:11.6° could not be used with high reliability because

of overlapping with a peak of polymorph B. All batches were measured. No traces of A could be detected. That means < 2% (see fig.2).

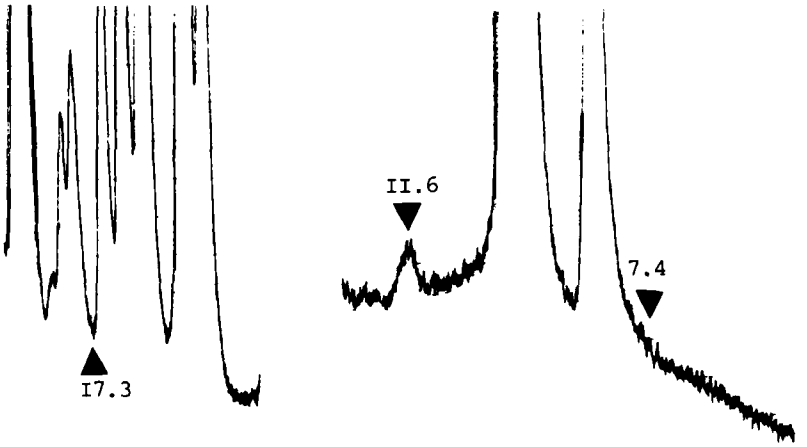

FIGURE 2 XRD scans of current batches : no peak at 7.4°

All given examples have been treated in the same way.

PROPYPHENAZONE

Propyphenazone may crystallize in 2 different crystalline modifications. Table II shows the suitability of 2 different peaks to quantify mixtures.

TABLE II Propyphenazone Standard deviations (s) and linearity 6 measurements for each concentration

Angle	50% B	25% B	20% B	15% B	10% B	5% B	r	a	b
11.8°	s:8%	s:12%	s:10%	s:11%	s:5.5%	s:16%	0.9946	2.7	-11
14.7°	s:7%	s:6%	s:13%	s:6%	s:10%	s:15%	0.9952	1.6	-2.6

X-ray diffraction allows us to quantify less than 5% form A and is able to demonstrate that some commercial batches contain form B as

described in previous experiments by means of DSC (1). In DSC experiments, heating rate and particle size are important factors to be considered in order to prevent artefacts of conversion of A into B (16). IR is unable to distinguish Forms A and B.

METOLAZONE

Metolazone exhibits polymorphism. The α-Form has an extremely low solubility and dissolution rate (1) and has to be limited in the commercial γ-Form.

Two peaks at 2θ:16.8° and 5.8° are characteristic of α-Form. The higher peak at 2θ:5.8° was chosen. The measurements given in table III show high variability at low concentrations but a detection limit of 1% is possible and the linearity reliable. Previous results

TABLE III Metolazone Linearity: 6 measurements for each concentration. Angle 5.8°. rate : 0.002°/s Range 10^3

% α	1 %	2 %	3 %	4 %	5 %	7 %	9 %	15%
Peak height	75	94	140	201	257	400	500	873
s %	22	20	26	17	19	15	11	7

Correlation coefficient r : 0.9965 Slope a : 59 Intercept b : -21

showed that DSC was unable to analyse α-Form due to kinetic transformation in the solid state (16). With IR the limit of detection was 10% (1).

CONTAMINANTS

XRD is able to detect contaminants. An example is the method for detecting asbestos in Talc (12). Using XRD for quantifying polymorphism we detected an unknown peak, due to a contaminant, whose position differed only 0.2° 2θ from the peak of the polymorph to be quantified. The batch passed all other methods of the control procedure.

STUDY OF POLYMORPHIC CHANGE DURING STORAGE

A new drug substance exists in 2 different crystalline modifications, A and B, exhibiting different dissolution rates. Two batches of Form A were stored according to a stability program. Only batch 2 contained traces of B. After storage, batch 1 remains unchanged but batch 2 transformed partially to form B. We compared abilities of DSC, IR and XRD to quantify this transformation.

DSC was able to distinguish both forms. The traces of lower melting form B in batch 2 were initially detected by DSC. DSC of mixtures, ground manually, give 2 peaks (162 and 166° C), incompletely resolved at level $\geq$5 % but separate till 50 %. Samples of batch 2, transformed to 20 %- 50 % after storage, give only one, broad peak overlapping both peaks of the 2 forms.

This behaviour demonstrates possible artefacts in DSC: mechanical mixtures do not behave like real mixtures of A and B. A transformation B $\rightarrow$ A probably occurs during melting.

Both IR and XRD were able to analyse mixtures. IR spectra and XRD of both forms are given in figures 3 and 4. Only IR in bands at 815 and 930 cm^{-1} could be used for quantification. The band at 2220 cm^{-1} remains

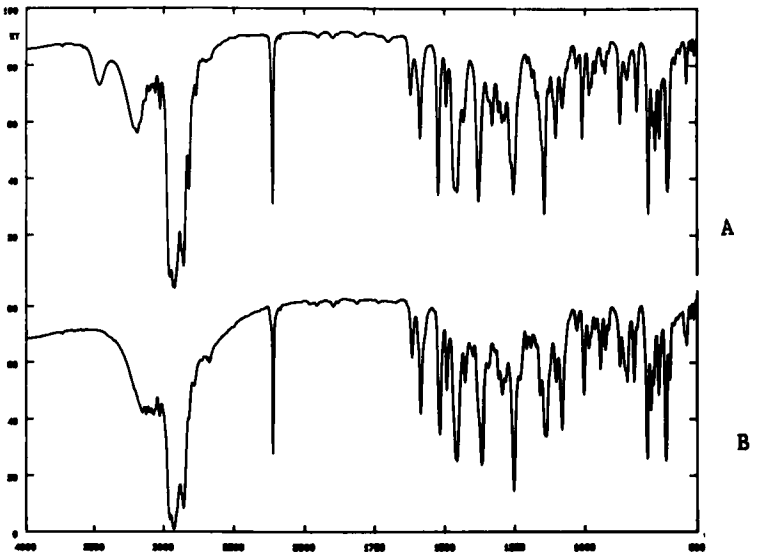

FIGURE 3 IR specta of forms A and B

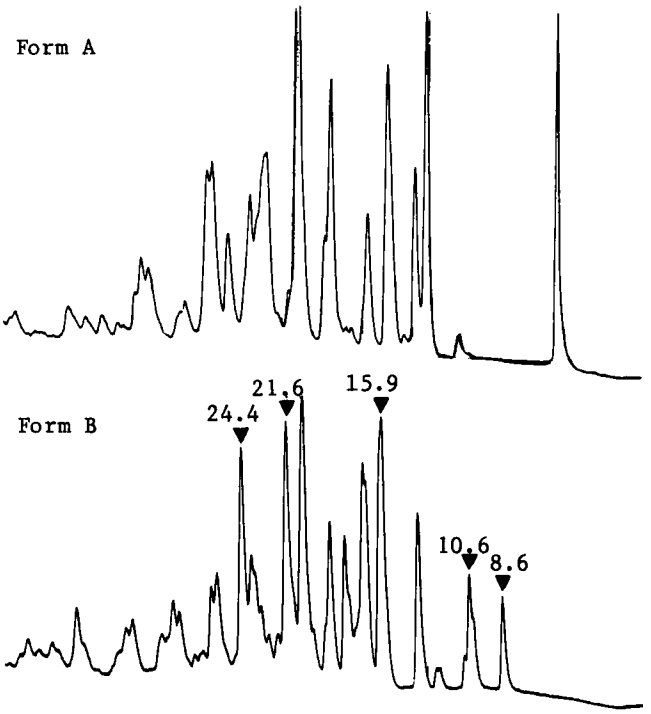

FIGURE 4 XRD of forms A and B

unchanged for both forms. The peak ratios 815 cm^{-1}/2220 cm^{-1} and 930 cm^{-1}/2220 cm^{-1} were calculated. Amounts lower than 15 %B cannot be accurately determined. Standard deviation of measurements lies at about 10 %. The XRD peak at 5.2° 2θ is useful for quantifying form A, and peaks at 2θ:8.6°, 10°6 and 21°6 could be used for calculation of form B.

Table IV summarises the standard deviations obtained for mixtures of 5 to 50 %B for each peak. Only the peak at 2θ:8.6 allows us to determine small amounts of form B.

TABLE IV Standard deviations of measurements of different concentrations at different angles (6 measurements)

Angle	50% B	40% B	30% B	20% B	10% B	5% B
5.2°	6.5%	5.7%	14.6%	9.9%	6.6%	not measurable
8.6°	5.5%	4.8%	3.4%	3.3%	6.6%	12.6%
10.6°	1.9%	1.8%	3.7%	1.7%	8.4%	not measurable
21.6°	2.6%	2.3%	6.3%	3.3%	3.3%	not measurable
24.4°	6.3%	5.8%	12.4%	11.3%	not measurable	

Table V gives results of linearity for XRD and IR. The best IR peak is at 930 cm^{-1} and the best peak for XRD is 2θ 8.6°. Peak 2θ, 5.2° is better for quantitative determination of A.

TABLE V Linearity for quantifying B in mixtures A + B in the range 10-50% for XRD and IR (n=6 for each concentration)

X-ray diffraction					
Angle	5.2°	8.6°	10.6°	21.6°	24.4°
Range	$2x10^4$	$5x10^3$	$5x10^3$	$1x10^4$	$1x10^4$
Correlation r	0.9923	0.9992	0.9991	0.9975	0.9872
Slope a	6.9	5.9	10.8	8.6	9.1
Intercept	-20	10	54	44	-107

Infra-red spectrum		
	Ratio 815/2220 (A)	Ratio 930/2220 (B)
Correlation coefficient	0.98852	0.97613
Slope	170	72
Intercept	4	3

Figure 5 shows IR at 930 cm^{-1} and XRD at 2θ = 8.6° of batch 2 after storing for 6 months, 1 year and 3 years at 30°C.

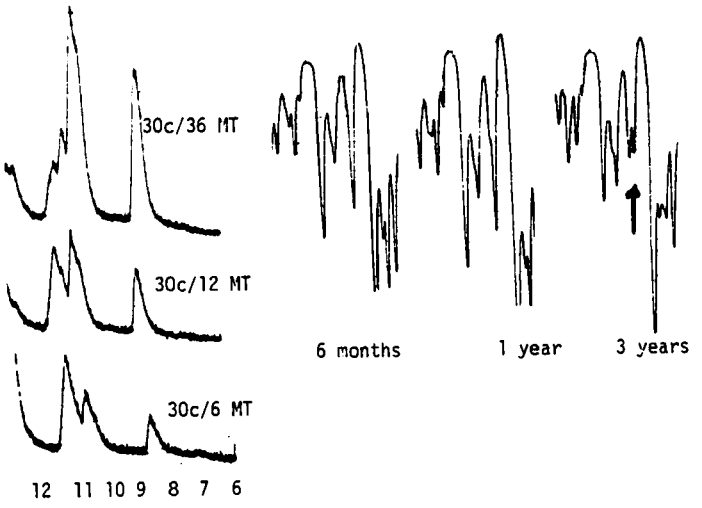

FIGURE 5 XRD of batch 2 at 30°C IR of batch 2 at 30°C

Table VI summarizes the study of polymorphic change in batch 2.

TABLE VI Study of polymorphic change during storage(TC=30°C/75%RH)
Results of Form B content after storage of batch 2

	Storage Time	-25°C	21°C 6 M	21°C 2 Y	30°C 6 M	30°C 1 Y	30°C 2 Y	30°C 3 Y	T.C 5 Y	50°C 1 Y
XRD	8.6°	5%	6%	16%	11%	18%	26%	39%	50%	22%
	10.6°	4%	16%	18%	9%	18%	30%	45%	55%	26%
	21.6°	5%	8%	16%	12%	22%	32%	52%	65%	25%
IR	815/2220	-	15%	11%	15%	22%	34%	52%	66%	35%
	930/2220	-	18%	21%	23%	19%	35%	66%	64%	27%

Figure 6 gives the results of measurements for transformation A → B after storage at 30 °C. XRD results are more reliable considering the standard deviation of measurements.

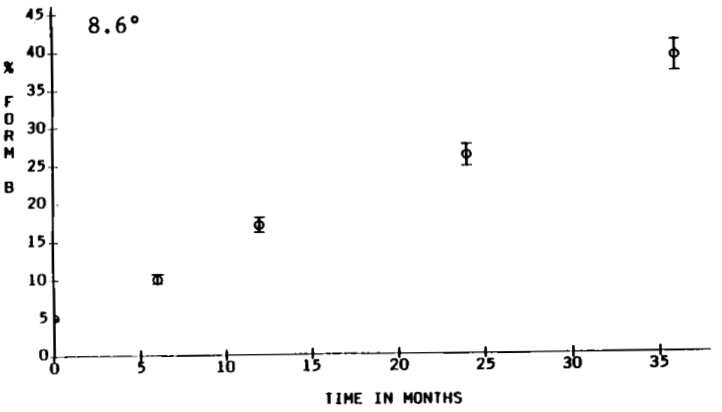

FIGURE 6 Plot of content of B in batch 2 versus time at 30°C

Such an XRD study of polymorphic transformation on storage has been done for phenylbutazone (17).

This case shows the need for a low quantification limit for polymorphism determination. Batch 1 did not contain any traces of form B and was unchanged after storage, whilst batch 2 containing approx. 5 % of form B transformed up to 50 % in B in the same storage

conditions. Only XRD was able to attain the quantification limit of 2%.

DOSAGE FORM

Figure 7 demonstrates the use of X-ray for monitoring the stability of a 10 % cream of a drug substance exhibiting polymorphism. The crystalline form is unchanged after storage.

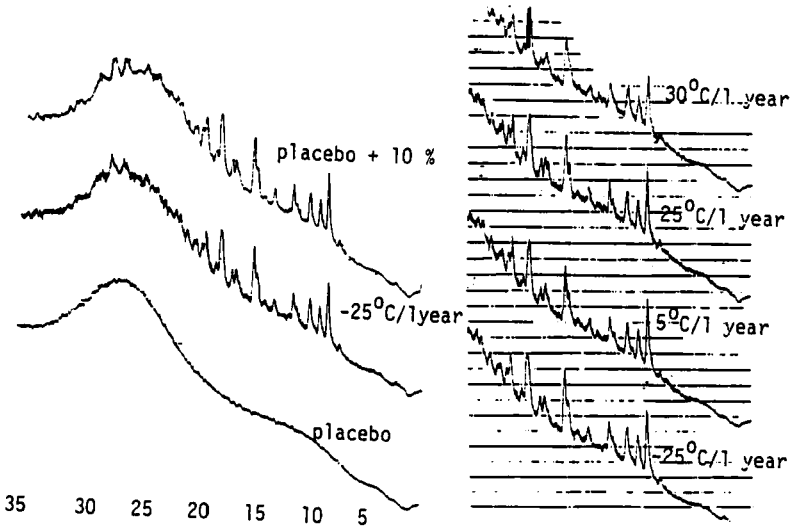

FIGURE 7 XRD of a cream, XRD of the cream after storage

Table VII deals with a 5 % dilution of a drug subtance in starch. The linearity is good and results of current batches are within content uniformity values, demonstrating the excellent performance of XRD for quantification of the drug substance in the matrix. (Angle 2θ 7.1°.)

TABLE VII XRD quantification of a new drug substance in 5% dilution with starch.

% Drug substance	1%	4%	7%	10%	batch 1	batch 2
deviation %	13%	5%	4%	0%	4% 3%	1% 5%
Results					5.1%/5.0%	5.2%/5.0%

r= 0.999 slope a = 39 intercept b = -3

CONCLUSION

In conclusion XRD quantification of pharmaceuticals is possible. The precision and reliability of the results depends on the validation of the method. Very low limits of quantification is necessary, and may be attained, in order to monitor batch to batch reproducibility and to study aging consequences.

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