of the 75% form I sample. Ten aliquots of this sample were packed and scanned over the range 2θ = 5–37°. The calculated mean was 76.7% form I. All 10 assays were within the 74–79% range. The mean difference was 2.1%, and the *SD* and *CV* were 1.8 and 2.3%, respectively. When zinc oxide was not used as an internal standard and the peak height ratio, $I_{25.1}/I_{19.8}$, was used, the calculated mean percentage of form I for these samples was 55%, and the range was 49–61%. Incorporation of zinc oxide as the internal standard substantially increased the accuracy and precision of the assay method. The uncertainty in the ratios in Table II is ~5%. This value is higher than the *SD* obtained from the repetitive testing of the 75% form I sample (Table II), 1.8%, and may be only a statistical anomaly.

In Table III are summarized the data obtained from the analysis of nine different mixtures of *N*-(4-hydroxyphenyl)retinamide. The mean difference for all nine mixtures was 2.3%. The largest differences between the known and calculated amounts of form I were −3 and +6%.

In summary, this method represents a precise and accurate method, within ±6%, for quantitation of the amount of *N*-(4-hydroxyphenyl)retinamide polymorph I present in samples of drug substance. The amount of form II can be calculated by difference. It appears that zinc oxide merits consideration for use as an internal standard in quantitative X-ray diffraction methods for other organic compounds.

REFERENCES

(1) R. C. Moon, H. J. Thompson, P. J. Becci, C. J. Grubs, R. J. Gander, D. L. Newton, J. M. Smith, S. L. Phillips, W. R. Henderson, L. T. Mullen, C. C. Brown, and M. B. Sporn, *Cancer Res.*, **39**, 1339 (1979).

(2) A. J. Aguiar, J. Krc, A. W. Kinkel, and J. C. Samyn, *J. Pharm. Sci.*, **56**, 847 (1967).

(3) S. A. Khalil, M. A. Moustafa, A. R. Ebian, and M. M. Motawi, *J. Pharm. Sci.*, **61**, 1615 (1972).

(4) T. Y. Yokoyama, T. Umeda, K. Kuroda, T. Nagafuku, T. Yamamoto, and S. Asada, *Yakugaku Zasshi*, **99**, 837 (1979).

(5) Y. Kato and M. Kohketsu, *Chem. Pharm. Bull.*, **29**, 268 (1981).

(6) Y. Kato and F. Watanabe, *Yakugaku Zasshi*, **98**, 639 (1978).

(7) W. D. Walkling, H. Almond, V. Paragamian, N. H. Batuyios, J. A. Meschino, and J. B. Appino, *Int. J. Pharm.* **4**, 39 (1979).

(8) G. J. Papariello, H. Letterman, and R. E. Huettemann, *J. Pharm. Sci.*, **53**, 663 (1964).

(9) D. B. Black and E. G. Lovering, *J. Pharm. Pharmacol.*, **29**, 684 (1977).

(10) W. E. Oberhaansli, H. P. Wagner, and O. Isler, *Acta Crystallogr. Sect. B*, **30**, 161 (1974).

(11) M. M. Thackery and G. Gafner, *Acta Crystallogr. Sect. B*, **31**, 335 (1975).

(12) H. Schenk, *Acta Crystallogr. Sect. B*, **27**, 667 (1971).

(13) M. M. Thackery and G. Gafner, *Acta Crystallogr. Sect. B*, **30**, 1711 (1974).

(14) H. Schenk, R. T. Kops, N. van der Putten, and J. Bode, *Acta Crystallogr. Sect. B*, **34**, 505 (1978).

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Absolute Intramuscular, Oral, and Rectal Bioavailability of Alizapride

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Received June 29, 1983, from the *Laboratoire de Biochimie I et de Pharmacologie, Centre Hospitalier Intercommunal de Créteil, 94000 Creteil, France.* Accepted for publication October 24, 1983.

Abstract □ A study was designed to estimate the absolute bioavailability of alizapride after intramuscular injection, oral administration as a solution or a tablet, and rectal administration as a suppository compared with that after intravenous injection. A balanced incomplete block-design trial was adopted. The intramuscular injection and the tablet administration showed identical results with those of the intravenous injection. On the contrary, the oral solution and the rectal suppository dosage forms gave lower absorption values, *i.e.*, 75 and 61% of the dose administered was absorbed, respectively.

Keyphrases □ Alizapride—bioavailability, intravenous, intramuscular, oral tablet, oral solution, rectal suppository □ Bioavailability—alizapride, intravenous, intramuscular, oral tablet, oral solution, rectal suppository □ Pharmacokinetics—alizapride, intravenous, intramuscular, oral tablet, oral solution, rectal suppository

Alizapride[1], *N*-[(1-allyl-2-pyrrolidinyl)methyl]-6-methoxy-1*H*-benzotriazole-5-carboxamide (I), is a new drug with antiemetic properties (1–3). It is used mainly in emergencies and is given intravenously in cancer patients (4), the pediatric population (5), and for internal medicine purposes (6). Chronic administration can be continued by intravenous, rectal, or even the oral routes of drug administration if patients are monitored well. For all these pharmaceutical forms, bioavailability results must be known to choose the appropriate dosage regimen for each route of drug administration.

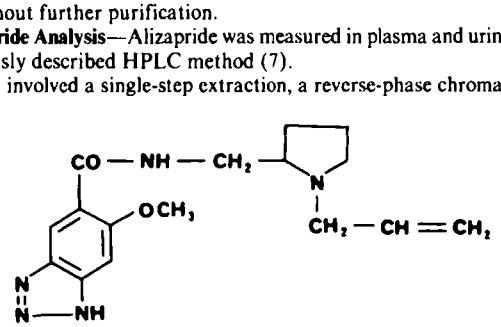
In a previous study (7), it has been shown that the pharmacokinetics of alizapride were independent of the dose administered, in the dosage range of 50–200 mg, either by the intravenous or oral routes. In this report are described the pharmacokinetic results obtained after intramuscular, oral solution or tablets, and rectal administrations compared with those after intravenous injection.

EXPERIMENTAL SECTION

Materials—Alizapride was obtained commercially and showed no impurities in two different TLC systems. All reagents for alizapride analysis in biological materials were of commercially available analytical grade and were used without further purification.

Alizapride Analysis—Alizapride was measured in plasma and urine by using a previously described HPLC method (7).

HPLC involved a single-step extraction, a reverse-phase chromatographic



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[1] Plitican; Delagrange Laboratories, Paris, France.

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Table I—Experimental Design for Alizapride Administration in 10 Subjects [a]

Subject	Form of Administration				
	Intravenous	Intramuscular	Oral Tablet	Oral Solution	Rectal
A	3	1	—	2	—
B	2	3	—	—	1
C	—	3	1	2	—
D	2	1	3	—	
E	—	2	—	1	3
F	3	—	2	—	1
G	1	—	2	3	—
H	—	3	1	—	2
I	1	—	—	2	3
J	—	—	1	3	2

[a] Numbers refer to the order of the forms administered separated by weekly intervals.

separation[2], and fluorometric detection[3]. Under the described conditions, the assay sensitivity was 5 ng/mL in plasma and 0.1 mg/L in urine, with a mean coefficient of variation of 3.9% between 25 and 2000 ng/mL. No endogenous interfering peaks appeared during the assays.

Alizapride Administrations—Because of the five alizapride dosage forms to be tested, a balanced incomplete block design (8) was adopted to avoid a complete block design, which would have led to a very cumbersome protocol. Ten subjects gave informed consent to participate in the study. They were free from cardiac, renal, hepatic, and respiratory diseases and allergies by clinical and biological examinations. None of the subjects received any drugs for at least 15 d prior to the study. Each subject received the same dose of 100 mg in three different forms at intervals separated by 1 week. The order of the pharmaceutical form of drug administration was done in a random, balanced fashion so that each form could be administered at least once. The experimental design for the drug administrations is indicated in Table I, with the numbers indicating the order of drug administration. All drugs were administered after an overnight fast, and oral tablets were administered with 200 mL of water. Subjects were allowed to have a light meal 4 h after drug administration.

Sampling—A 7-mL heparinized blood sample was withdrawn at 0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min and 3, 4, 6, 8, 10, 12, and 24 h after drug administration. Urine samples were collected every 2 h during the first 12 h, once during the next 12 h, and every 24 h during the next 4 d.

Calculations—Statistical and pharmacokinetic calculations were performed with a table microcomputer[4]. Pharmacokinetic parameters were obtained with previously developed programs (7, 9). The interpretation of drug concentrations as a function of time was systematically performed according to three different pharmacokinetic models, *i.e.*, one-, two-, or three-compartment open models by a Gauss–Newton algorithmic method. At each step, a statistical Fischer test with the least-squares criterion was used to evaluate the benefit of increasing the number of compartments. Pharmacokinetic parameters were then calculated by the equations of Wagner (10). Results were compared for the different pharmaceutical forms using an analysis of variance (ANOVA) adapted to the trial design (11, 12). Furthermore, the symmetrical confidence intervals were estimated for the comparisons of the area under the curves and the amounts excreted unchanged in the urine after each administered form (8, 13).

RESULTS AND DISCUSSIONS

Intravenous Administration—The mean alizapride concentrations obtained from the six subjects receiving the intravenous form are shown in Figs. 1 and 2. The corresponding mean pharmacokinetic parameters estimated from in-

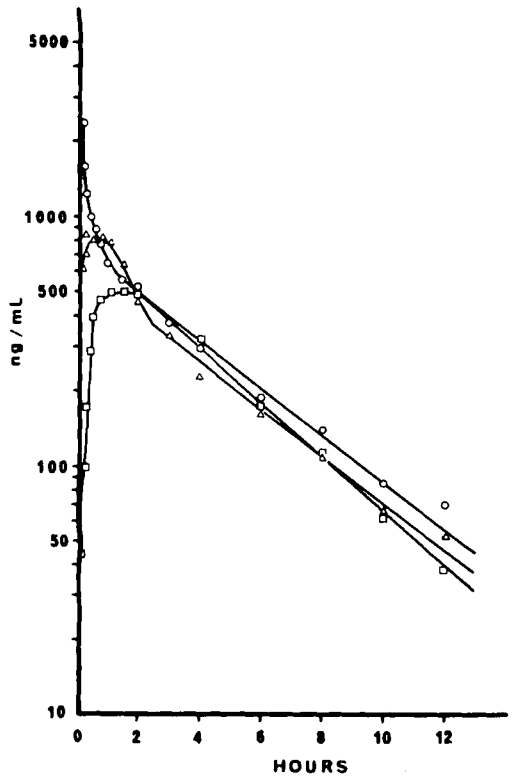

Figure 1—*Mean plasma concentrations of alizapride* versus *time obtained from six subjects after intravenous, intramuscular, or rectal route of administration. Key: (○) intravenous; (△) intramuscular; (□) rectal.*

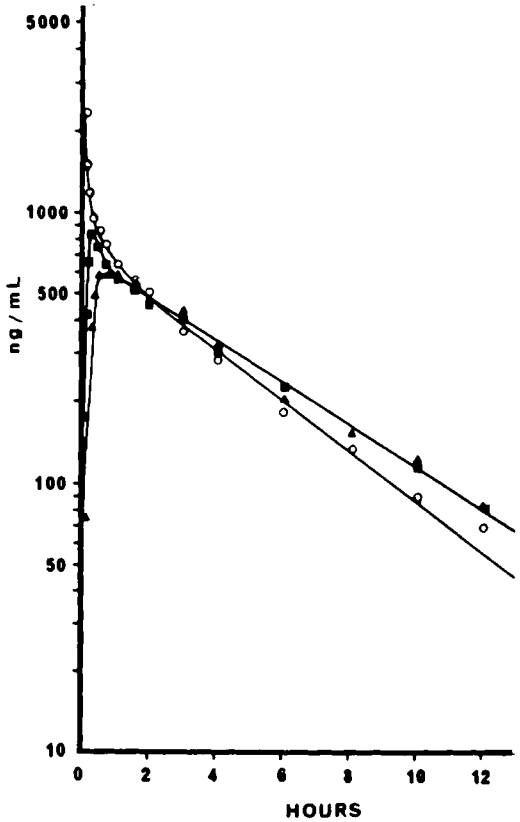

Figure 2—*Mean plasma concentrations of alizapride* versus *time obtained from six subjects after intravenous and oral administration as tablet or solution. Key: (○) intravenous; (▲) tablet; (■) solution.*

[2] Bondapak C_{18}; Water Associates, Paris, France.
[3] JY3; Jobin et Yvon, Paris, France.
[4] Model 4051; Tektronix, Paris, France.

Table II—Mean Values of the Pharmacokinetic Parameters Obtained after Administration of Different Pharmaceutical Forms of Alizapride

Parameter	Alizapride Administration, 100 mg				
	Intravenous	Intramuscular	Oral Tablets	Oral Solutions	Rectal
Lag time, h	—	0.00	0.18 ± 0.15	0.09 ± 0.05	0.17 ± 0.10
K_r, h^{-1}	—	7.13 ± 5.56	6.54 ± 4.82	12.10 ± 10.54	5.21 ± 6.36
$t_{1/2r}$, h	—	0.13 ± 0.06	0.20 ± 0.17	0.13 ± 0.16	0.44 ± 0.48
$t_{1/2\alpha}$, h	0.10 ± 0.05	0.48 ± 0.16	—	0.22 ± 0.07	—
$t_{1/2\beta}$, h	2.75 ± 0.78	2.04 ± 0.51	3.10 ± 0.63	2.68 ± 0.68	2.55 ± 1.24
k_{13}, h^{-1}	0.88 ± 0.54	0.348 ± 0.100	0.234 ± 0.060	0.272 ± 0.063	0.340 ± 0.183
k_{12}, h^{-1}	4.81 ± 2.59	0.66 ± 0.12	—	1.55 ± 0.75	—
k_{21}, h^{-1}	2.14 ± 0.54	0.76 ± 0.64	—	1.41 ± 0.68	—
V_1, L	36.3 ± 22.6	90.4 ± 18.7	112.6 ± 25.4	117.0 ± 59.4	150.3 ± 81.4
V_2, L	61.4 ± 11.3	55.7 ± 31.3	—	65.4 ± 15.2	—
AUC, mg·h/L	3.80 ± 0.62	3.62 ± 1.63	3.53 ± 1.08	2.86 ± 1.24	2.32 ± 0.69
CL, mL/min	461.8 ± 57.3	517.1 ± 160.2	505.6 ± 129.5	585.0 ± 265.0	670.2 ± 161.3
CL_R, mL/min	372.0 ± 58.0	422.5 ± 140.7	397.0 ± 108.8	396.2 ± 142.2	425.0 ± 171.5
U_∞, mg	80.5 ± 5.4	81.4 ± 8.6	78.3 ± 7.3	69.7 ± 7.1	61.8 ± 9.8

dividual results are presented in Table II. The results show a rapid distribution phase corresponding to a half-life of 0.10 ± 0.05 h (range, 0.062–0.15 h) and an elimination phase with a mean half-life of 2.75 ± 0.78 h (range, 1.75–3.99 h). The volumes of distribution were large [36.3 ± 22.6 L (range, 14–68 L) for the central compartment and 61.4 ± 11.3 L (range, 44.1–72.8 L) for the peripheral compartment]. The total clearance of elimination was moderate [461.8 ± 57.3 mL/min (range, 405.5–533 mL/min)]. The mean unchanged drug urinary elimination curves of the six subjects are shown in Fig. 3. The overall urinary elimination of unchanged alizapride (80.51 ± 5.4%) of the dose administered (range, 73.2–88.7%) indicates that urinary excretion is the main route of alizapride elimination. About 20% of the injected dose was not recovered in this study. This cannot be explained by an insufficient amount of urine collected (Fig. 3). So the difference must be accounted for by metabolism or another route of elimination of unchanged drug or its metabolites, such as biliary excretion. It is noteworthy that in a previous study (7) it was shown that the chromatographic method was specific for the unchanged drug, both in urine and plasma, and no extra peak appeared in the chromatograms. Therefore, from the data of alizapride urinary elimination, the renal clearance can be calculated to be 372.0 ± 58.0 mL/min, indicating an active tubular process of alizapride excretion.

Intramuscular Administration—The plot of mean plasma concentrations of alizapride *versus* time is shown in Fig. 1, and the corresponding pharmacokinetic parameters are presented in Table II. The appearance of the drug in plasma is very rapid, corresponding to a rate constant of 7.13 ± 5.56 h^{-1}. Maximum concentrations were 848 ± 193 ng/mL and occurred within 20 min after injection. The calculated parameters were very close to those observed after intravenous injection. It is noteworthy that the area under the plasma concentration curves (AUC) and the amount excreted unchanged in the urine are identical.

Oral Administration—The plot of the mean plasma concentrations of alizapride *versus* time obtained after the oral administration of tablets and solutions are shown in Fig. 2. The corresponding mean pharmacokinetic parameters are listed in Table II. The maximum plasma concentrations were higher after solution administration than after tablet administration (mean, 930 ± 350 ng/mL *versus* 740 ± 90 ng/mL) and occurred slightly sooner after solution administration (0.57 ± 0.34 h *versus* 0.69 ± 0.47 h). Similarly, the lag time observed with the solution dosage form was two times lower than that of the tablet. Compared with the parameters obtained after the intravenous injection, the AUC values were lower (mean, 3.53 ± 1.08 and 2.86 ± 1.24 mg·h/L, respectively, for the tablet and the solution), and the amount excreted unchanged in the urine decreased to 78.3 ± 7.3 and 69.7 ± 7.1 mg for the tablets and solutions, respectively. With these dosage forms, therefore, the bioavailability seemed to decrease, resulting in increased apparent volumes of distribution and total clearances, since these parameters were estimated on the assumption of total absorption. On the contrary, the elimination half-lives and the renal clearances, which are independent of the percentage of the dose administered that was absorbed, are identical with those obtained after intravenous injection.

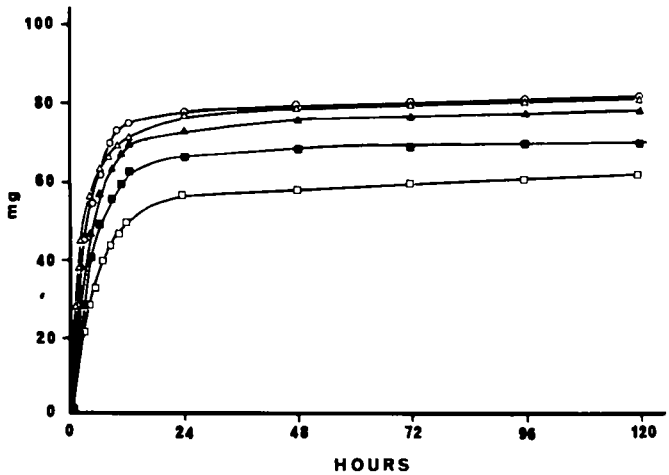

Figure 3—*Mean unchanged alizapride urinary elimination curves obtained from six subjects after each of the different forms was administered. Key: (○) intravenous; (△) intramuscular; (▲) tablet; (■) oral solution; (□) rectal.*

Rectal Administration—The plots of the mean plasma and urine concentrations *versus* time are shown in Figs. 2 and 3, respectively. The corresponding pharmacokinetic parameters are listed in Table II. The maximum plasma concentrations obtained with suppositories were the lowest (630 ± 210 ng/mL) and were seen last at 1.26 ± 1.02 h after administration. The amounts absorbed from this form seemed to be low since the AUC was 2.32 ± 0.69 mg·h/L and the mean urinary elimination was 61.8 ± 9.8 mg. The mean elimination half-lives and renal clearances were identical with those observed after intravenous injection.

Bioavailability Study—The absolute bioavailability of the four dosage forms was estimated from the AUC and the overall amounts of unchanged excreted drug. The ANOVA analyses corresponding to the trial design showed no statistical differences at the 0.05 levels between subjects or time of drug administration for both parameters.

The AUC ANOVA failed to show an overall difference between the dosage forms ($^4_{16}F$ = 2.996, but was very close to the significant value at the 0.05 level, ($^4_{16}F$ = 3.01). However, for the urinary excretion studies, ANOVA showed a significant difference in the tested form at the 0.01 level ($^4_{16}F$ = 5.47). The protected multiple Student t test (11), applied for comparisons of each dosage form against intravenous injections, showed that intramuscular and oral tablet administrations were not significantly different from the intravenous administration (t = 0.9 and 2.25, respectively). However, the oral solution and the rectal suppositories both differed significantly from the intravenous injection at the 0.01 level (t = 10.8 and 18.7, respectively).

The difference observed between the two parameters can be explained by the greater variability observed with the AUC. This is shown by the estimation of the symmetrical confidence interval according to Westlake (8, 13) which yielded 36.9 and 31.2% intervals for the tablet and the intramuscular forms when estimated from AUC and only 13.0 and 13.9% for the same forms, respectively, when estimated from amounts excreted in the urine. Due to this greater variability, the ANOVA was unable to show any difference, although it seemed to occur.

It was surprising to find that the oral solution showed significantly lower bioavailability than did the tablet forms since, in most cases, solutions yielded higher bioavailabilities than did the solid forms. Such an observation has been made with two barbiturates, namely, cyclobarbital (14) and heptabarbital (15). Physiological explanations for such a phenomenen are difficult. Either a precipitation in the stomach and/or the intestinal tract or a delay in the transfer of the drug from the stomach to the intestine can occur (10). The latter was unlikely to have occurred with alizapride since it appeared very rapidly in the blood stream with both oral forms, and the maximum plasma concentrations were obtained very rapidly.

Differences in the rate of absorption of the drug with the extravascular forms were tested by using the nonparametric Kruskal–Wallis test (11) for

the observed plasma maximum concentrations and the time at which they occurred. This test failed to show any difference between the four tested forms with the two chosen parameters. Therefore, the study of the bioavailability of intramuscular, oral solution, tablet, and rectal suppository administration compared with intravenous administration showed that the intramuscular dosage form and oral tablets gave results similar to those obtained by intravenous injection, but the oral solution and the suppositories showed a mean lower bioavailability of 75 and 61%, respectively.

REFERENCES

(1) P. Casanova, *Hepatology*, **16**, 381 (1980).

(2) J. M. Segrestua, J. Gueris, D. Julien, and M. Tiar, *Thérapie*, **34**, 437 (1979).

(3) C. Laville and J. Margarit, *Sem. Hôp. Paris*, **58**, 323 (1982).

(4) E. Pommat, *Sem. Hôp. Paris*, **58**, 363 (1982).

(5) F. Battistelli and H. Lestradet, *Sem. Hôp. Paris*, **58**, 349 (1982).

(6) D. Girard, E. Salles, and A. Bel, *Sem. Hôp. Paris*, **58**, 353 (1982).

(7) G. Houin, F. Bree, N. Lerumeur, and J. P. Tillement, *J. Pharm. Sci.*, **72**, 71 (1983).

(8) W. J. Westlake, in "Principles and Perspectives in Drug Bioavailability," J. Blanchard, R. J. Sawchuk, and B. B. Brodie, Eds., Karger, Basel, 1979, chap. 1.

(9) P. D'Athis, M. O. Richard, D. Delauture, E. Rey, M. Bouvier-d'Yvoire, E. Clément, and G. Olive, *Thérapie*, **36**, 443 (1981).

(10) J. G. Wagner, in "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1975.

(11) W. G. Cochran and G. M. Cox, in "Experimental Designs," Wiley, New York, N.Y., 1957.

(12) W. J. Westlake, *Biometrics*, **30**, 319 (1974).

(13) W. J. Westlake, *Biometrics*, **32**, 741 (1976).

(14) D. D. Breimer, *J. Pharm. Sci.*, **64**, 1576 (1975).

(15) D. D. Breimer and A. G. de Boer, *Eur. J. Clin. Pharmacol.*, **9**, 169 (1975).

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Kinetic Study of the Polymorphic Transformations of Phenylbutazone

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Received June 15, 1983, from the *Kobe Women's College of Pharmacy, Higashinada, Kobe 658, Japan.* Accepted for publication December 5, 1983.

Abstract □ The polymorphic transformations of phenylbutazone from metastable forms α and β to stable form δ were studied quantitatively at four temperature and five humidity levels by X-ray powder diffractometry. The transformation of form α conformed with the Avrami-Erofe'ev kinetic model and form β conformed with apparent first-order kinetics. In the two transformation systems, the induction periods depended on the storage conditions and were prolonged with lowering of temperature and humidity. The transformation rate of form α was not affected by humidity, whereas that of form β increased according to a rise in humidity. The temperature dependency of the transformation rate constant was remarkable. The Arrhenius treatment was applicable to the $\beta \rightarrow \delta$ transformation at low temperatures. The overall half-life, including induction period, revealed that form α was more stable than form β under any storage condition. A good linear relationship existed between the induction period and the transformation rate constant, irrespective of the storage conditions. The scanning electron photomicrographs of forms α and β demonstrated that acicular crystals of form δ grew as the transformation progressed. This could be confirmed as the change in particle diameter of the samples.

Keyphrases □ Phenylbutazone—polymorphic transformation, physicochemical stability under different storage conditions □ Polymorphic transformation—phenylbutazone, physicochemical stability under different storage conditions □ Physicochemical stability—phenylbutazone, different storage conditions, polymorphic transformation

The method for improving the bioavailability of a slightly soluble drug is an important problem in the preformulation study of solid dosage forms. Polymorphic transformation is often effective as a technique for increasing solubility. Since the solubility and dissolution rate of a metastable polymorph are usually higher than those of the stable form, the former is more desirable clinically than the latter; however, the former is not always preferable in physicochemical stability. It is well known that, irrespective of temperature, only one crystal form is thermodynamically stable and all other forms convert eventually to the stable one. A metastable form nevertheless may occasionally exhibit sufficient stability to ensure a reasonable shelf life. If a metastable form is used in a formulation because of its excellent dissolution properties, it is prerequisite to demonstrate that the metastable form is never transformed to a more stable form under usual storage conditions.

Although some difficulties are involved in establishing a quantitative method for polymorphic transformation, there are several previous reports on the method (1–8). Due to the complexity of solid-state reaction, the stability-indicating kinetic interpretation has been fully discussed in only a few reports (1, 7, 8). The techniques described here are divided into three groups: IR spectrophotometry based on the quantitative Nujol mull technique (1–3, 6), differential scanning calorimetry (4, 5), and X-ray powder diffractometry (7, 8). Among these, X-ray diffractometry may be the most useful method because heat and liquid relating closely to polymorphic transformation are not involved in the measurement.

In the present investigation, the stability profiles between the two metastable forms of phenylbutazone are comparatively evaluated. The preparation and characterization of the phenylbutazone polymorphism have been described previously (9–16). The polymorphic system has been reported to consist of a stable form (form δ), four metastable forms (forms α, β, γ, and ϵ), and two pseudopolymorphs, among which form ϵ has the highest solubility. For the reasons of high yield and simplicity of preparation, forms α and β were chosen as polymorphic models in this study. The transformations of these forms were measured quantitatively by X-ray powder diffractometry and thoroughly studied over long periods of time under different storage conditions.

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