Analysis of N-(4-Hydroxyphenyl)retinamide Polymorphic Forms by X-ray Powder Diffraction

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Received December 6, 1982, from the *Department of Chemical and Pharmaceutical Development, McNeil Pharmaceutical, Spring House, PA 19477.* Accepted for publication November 16, 1983.

Abstract □ A quantitative X-ray diffraction method, with zinc oxide used as the internal standard, was developed for the analysis of polymorphic forms I and II of *N*-(4-hydroxyphenyl)retinamide. The standard curve relating peak height ratio to the percentage of form I was linear. The method was precise and accurate to within ±6%.

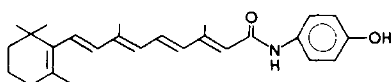
Keyphrases □ *N*-(4-Hydroxyphenyl)retinamide—X-ray powder diffraction analysis of polymorphic forms □ X-ray powder diffraction—analysis of polymorphic forms of *N*-(4-hydroxyphenyl)retinamide □ Polymorphs—*N*-(4-hydroxyphenyl)retinamide, analysis by X-ray powder diffraction

N-(4-Hydroxyphenyl)retinamide (fenretinide; III), an analogue of vitamin A which was first synthesized by Gander and co-workers (1), has a demonstrated prophylactic effect against mammary tumors in rats. Two polymorphic forms of *N*-(4-hydroxyphenyl)retinamide which occur alone or in combination after recrystallization have been identified by X-ray powder diffraction (Fig. 1).

The polymorphic form of a drug may affect its efficacy. The bioavailability of both chloramphenicol (2) and sulfameter (3) after oral administration in humans is dependent on their polymorphic forms. Studies in animals with polymorphs of indomethacin (4), amobarbital (5), and phenobarbital (6) have demonstrated the nonequivalence of polymorphic forms. To monitor the polymorphic character of *N*-(4-hydroxyphenyl)-retinamide drug substance used in preclinical animal testing and to evaluate the physicochemical properties of the two forms, a quantitative analytical procedure was developed.

X-ray powder diffraction and IR spectroscopy have been used for quantitative analysis of mixtures of polymorphs. Walkling *et al.* (7) have developed an IR spectroscopic method to quantitate difenoxin hydrochloride polymorph mixtures. Aguiar *et al.* (2) have used X-ray powder diffraction to determine the composition of chloramphenicol palmitate polymorph mixtures by calculating the peak height ratio of two peaks, with each peak characteristic of one of the two forms. The calibration line they obtained was curvilinear rather than straight. They found that the amorphous form did not interfere and that they could correct for the amount of a third crystalline form occasionally present. Papariello *et al.* (8) have successfully used X-ray powder diffraction as a nondestructive analytical method for measuring the amount of glutethimide in intact tablets. Black and Lovering (9) have used X-ray powder diffraction to quantitate the crystalline polymorphs in digoxin. X-ray diffraction studies of vitamin A analogues appear to have been limited to single-crystal studies to elucidate their chemical structures (10–14).

In this report is described a quantitative X-ray powder diffraction method which determines the amounts of the polymorphic forms I and II of *N*-(4-hydroxyphenyl)retinamide in samples of *N*-(4-hydroxyphenyl)retinamide drug substance. Zinc oxide was selected as the internal standard since all of its diffraction peaks (Fig. 1) occur at higher reflection angles than those of the two polymorphic forms of *N*-(4-hydroxyphenyl)-retinamide.



N-(4-hydroxyphenyl)retinamide

EXPERIMENTAL SECTION

Reagents and Materials—Zinc oxide[1] (internal standard), analytical reagent grade, was obtained commercially and was passed through an 80-mesh sieve prior to use. *N*-(4-Hydroxyphenyl)retinamide was ≥99% pure, as determined by an HPLC method in which the 13-*cis* isomer was separated from the all-*trans* isomer, *N*-(4-hydroxyphenyl)retinamide. There was <0.5% 13-*cis* isomer in the test materials. Mixtures of forms I and II gave the same HPLC peak and TLC spot. There were qualitative differences in the IR spectra of forms I and II that were suggestive of polymorphism. The solution NMR spectra were identical, indicating that the two forms were not isomers of each other. The two forms do not interconvert under the conditions described in this report.

Standards—Authentic samples of *N*-(4-hydroxyphenyl)retinamide forms I and II were passed through an 80-mesh sieve. When *N*-(4-hydroxyphenyl)-retinamide was passed through a 200-mesh sieve, the sample could not be used due to the induction of a static electrical charge. The 80-mesh powder samples were used as the 100 and 0% form I standards. The 25, 50, and 75% form I standards were prepared by mixing forms I and II in a mortar.

Assay—A sample (200 mg) of *N*-(4-hydroxyphenyl)retinamide or standard was mixed with 40 mg of zinc oxide in a glass mortar. The *N*-(4-hydroxyphenyl)retinamide–zinc oxide mixture (5:1) was placed into a sample holder, and the powder was pressed into place with a glass slide by moving the slide from side to side, thereby leaving a smooth, lightly compacted surface. The samples were scanned over the 5–37° 2θ range. The peak heights of the sample and internal standard were measured at 19.1° and 31.8° 2θ from a baseline drawn from 11° to 33° 2θ. The ratio $I_{19.1}/I_{31.8}$ was calculated, and the amount of form I was obtained from a standard curve relating $I_{19.1}/I_{31.8}$ *versus* the percentage of form I.

Apparatus—An X-ray diffractometer[2] containing a long fine-focus copper tube X-ray source, diffracted-beam monochromater, theta-compensating slit, and scintillation counter was used. The X-ray tube was operated at 40 kV and 30 mA. The scan speed was 1°/min; range, 5 kcps; time constant, 1. The takeoff angle was 6°.

Reproducibility and Accuracy—A 2.5-g mixture was prepared, as described above, containing 75% form I and 25% form II. The mixture was mixed with zinc oxide (5:1) and analyzed 10 times using a different aliquot for each assay. Nine other mixtures were prepared in which the amount of form I ranged from 19 to 100%. The concentrations of form I were determined for the 10 samples and compared with the known concentrations.

RESULTS AND DISCUSSION

The X-ray powder diffraction patterns for polymorphic forms I and II are shown in Fig. 1. The *d*-spacings and relative intensities of the two polymorphs are tabulated in Table I. It appears that a number of peaks such as 2θ = 19.1°, 25.1°, and 19.8° could be used quantitatively to measure the amounts of forms I and II in samples of drug substance. Since the peaks are sharp and have about the same width at half-height, peak height is a reasonable approximation of the area of the peak. Several peak height and peak height ratio combinations were evaluated. None were sufficiently accurate for determining the composition of known mixtures. The low angle peaks below 11° 2θ, at which

[1] Fisher Scientific Co.
[2] Philips Electronic Instruments, Inc.

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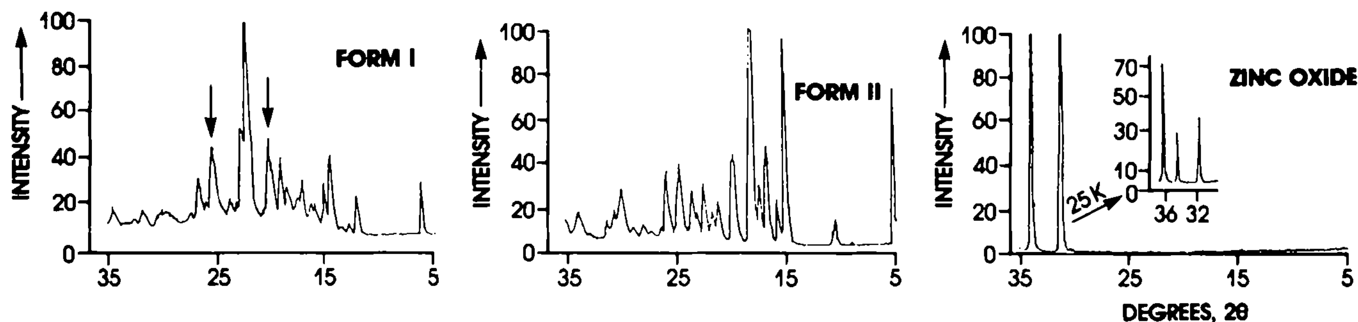

Figure 1—*X-ray diffraction patterns of polymorphic forms I and II and zinc oxide.*

Table I—Tabulated Diffraction Data from *N*-(4-Hydroxyphenyl)retinamide Forms I and II

Form I					Form II				
Line	2θ°	d-(nm)	I	I/I₀	Line	2θ°	d-(nm)	I	I/I₀
1	5.9	1.4966	30.5	19	1	5.3	1.6658	80.5	45
2	7.3	1.2098	3.5	2	2	9.0	0.9817	3	2
3	11.9	0.7430	22.5	14	3	10.5	0.8417	16	9
4	12.6	0.7019	22	14	4	10.8	0.8184	7	4
5	13.3	0.6651	11.5	7	5	15.1	0.5862	103.5	57
6	14.3	0.6188	68	42	6	15.7	0.5639	22	12
7	14.9	0.5940	71.5	44	7	16.8	0.5272	61	34
8	15.8	0.5604	36.5	23	8	17.4	0.5092	39	22
9	16.2	0.5466	20.5	13	9	18.1	0.4896	180.5	100
10	16.9	0.5241	42.5	26	10	19.7	0.4502	48	27
11	17.2	0.5151	37.5	23	11	19.9	0.4457	51.5	29
12	18.4	0.4817	39.5	24	12	21.1	0.4207	24.5	14
13	19.1	0.4642	80.5	50	13	21.6	0.4110	19	11
14	19.8	0.4480	65.5	40	14	22.5	0.3948	41	23
15	21.9	0.4055	162	100	15	23.0	0.3863	17.5	10
16	22.3	0.3983	72.5	45	16	23.5	0.3782	29	16
17	23.5	0.3782	29.5	18	17	24.6	0.3615	42	23
18	25.1	0.3544	77	48	18	24.8	0.3587	25	14
19	26.4	0.3373	37	23	19	25.9	0.3437	48	27
20	27.2	0.3275	12	7	20	26.4	0.3373	6.5	4
21	29.3	0.3045	12.5	8	21	27.2	0.3275	3	2
22	31.5	0.2837	10.5	6	22	28.0	0.3184	8.5	5
23	34.2	0.2619	11.5	7	23	28.8	0.3097	5	3
					24	29.9	0.2986	24	13
					25	30.6	0.2919	12	7
					26	30.9	0.2891	10	6
					27	31.2	0.2864	8	4
					28	33.9	0.2642	15.5	9

overlap was reduced, would appear to be good candidates for quantitation. Unfortunately, the intensities of the peak heights of each component were not always proportional to the amounts present.

Zinc oxide was selected as the internal standard because of the proximity of its major peak at $2\theta = 31.8°$ (100), 34.5° (002), and 36.3° (101) to the major peaks of the two polymorphic forms of *N*-(4-hydroxyphenyl)retinamide, and a complete absence of zinc oxide peaks in the region below $2\theta = 30°$. Form I has a small peak at $2\theta = 31.5°$ which did not interfere with the measurement of the zinc oxide peak, as evidenced by the good correlation and linearity of the calibration curve. The peak widths at $2\theta = 19.1°$ and 31.8° were between 0.2° and 0.4° when measured at half-height in all tested samples.

The calibration curve, relating the peak height ratio $I_{19.1}/I_{31.8}$ *versus* the percentage form I, was linear ($y = 0.0143 + 0.00259x$; $r = 0.9997$). A *y*-intercept occurred as a result of the method used to establish the baseline and the slight amount of background noise observed at $2\theta = 19.1°$ with 0% form I (100% form II) standards. The excellent correlation and linearity of the calibration curve indicate that the selection of zinc oxide as the internal standard was appropriate. An organic internal standard is usually preferred for the analysis of organic compounds; however, the number of peaks in the X-ray diffraction patterns of the two forms of *N*-(4-hydroxyphenyl)retinamide (Fig. 1) make it unlikely that an organic internal standard would have been suitable.

In Table II are summarized the results obtained from the repetitive testing

Table II—Precision of Analysis of *N*-(4-Hydroxyphenyl)retinamide in a Mixture of Known Composition [a]

Trial	$I_{19.1}/I_{31.8}$	Form I, %
1	0.219	79
2	0.211	76
3	0.215	78
4	0.204	74
5	0.212	76
6	0.206	74
7	0.213	76
8	0.215	78
9	0.220	79
10	0.214	77
	Mean	76.7
	Range	74–79
	Mean Difference	2.1
	SD	1.8
	CV	2.3

[a] 75% form I.

Table III—Analysis of Nine Mixtures

Mixture	Form I Percent Known	$I_{19.1}/I_{31.8}$	Form I Percent Calculated	Difference, % [a]
A	19	0.0564	16	−3
B	60	0.170	60	0
C	53	0.154	54	1
D	80	0.214	77	−3
E	69	0.189	68	−1
F	29	0.084	27	−2
G	90	0.256	93	3
H	100	0.280	106	6
I	40	0.113	38	−2

[a] Mean difference, 2.3%.

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.

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ACKNOWLEDGMENTS

The authors thank Mr. J. N. Plampin for providing authentic samples of *N*-(4-hydroxyphenyl)retinamide forms I and II, Mr. M. Mutter for providing NMR and IR spectra of the two forms of *N*-(4-hydroxyphenyl)retinamide, and Drs. W. D. Walkling and R. Jenkins for helpful suggestions during the preparation of this manuscript.

This study was presented at the 130th Annual Meeting of the American Pharmaceutical Association, New Orleans, La., April 1983.

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.

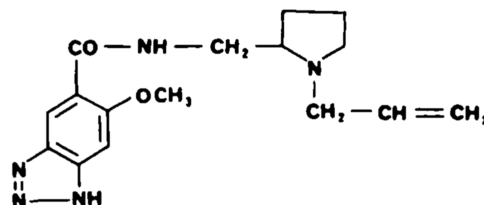
In a previous study (7), it has been shown that the pharmacokinetics of alizapride were independent of the dose administered, in the dosage range of 50–200 mg, either by the intravenous or oral routes. In this report are described the pharmacokinetic results obtained after intramuscular, oral solution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