(4) Potter, E., Jacobs, P., and Weber, J., unpublished data, Connaught Medical Research Laboratories, University of Toronto, Toronto, Ontario, Canada.
(5) Nash, T., *Biochem. J.*, 55, 416(1953).
(6) Taylor, E. M., and Moloney, P. J., THIS JOURNAL, 46, 299(1957).
(7) Jones, P. S., Thigpen, D., Morrison, J. L., and Richardson, A. P., *ibid.*, 45, 268(1956).
(8) Kellaway, C. H., MacCallum, P., and Tebutt, A. M., "Report of the Royal Commission of Inquiry into Fatalities at Bundaberg with Appendices," H. J. Green, Government Printer, Canberra, Australia, 1928.
(9) Baker, S. J., *Med. J. Australia*, I, 750(1960).
(10) Olin, G., and Lithander, A., *Acta Pathol. Microbiol. Scand.*, 25, 152(1948).
(11) Falk, C. R., and Applington, S., *Am. J. Hyg.*, 24, 285(1936).
(12) Simone, R. M., and Popino, R. T., THIS JOURNAL, 44, 275(1955).
(13) McLean, I. W., and Taylor, A. R., *Progr. Med. Virol.*, 1, 122(1958).
(14) Feldman, M. Ya., *Probl. Virol. U.S.S.R. Engl. Transl.*, 4, 54(1959).
(15) "Minimum Requirements: Poliomyelitis Vaccine," 1st rev., Amendment No. 4, Division of Biologic Standards, National Institutes of Health, Bethesda, Md., April 12, 1955.
(16) Salk, J. E., Krech, U., Younger, J. S., Bennet, B. L., Lewis, L. J., and Bazeley, P. L., *Am. J. Public Health*, 44, 563(1954).
(17) Cooper, E. A., and Mason, J., *J. Hyg.*, 26, 118(1927).
(18) Klein, M., *Trans. Ophthalmol. Soc. U. K.*, 74, 479(1954).
(19) Klein, M., Millwood, E. G., and Walther, W., *J. Pharm. Pharmacol.*, 6, 725(1954).
(20) Lowbury, E. J. L., *Brit. J. Ind. Med.*, 8, 22(1951).
(21) Ramon, G., and Richou, R., *Presse Med.*, 55, 693 (1947).
(22) Bray, M. D., U. S. pat. 2,728,705.
(23) Schmidt-Lorenz, W., *Lebenem. Untersuch. Forsch.*, 108, 423(1958).
(24) Edsall, G., McComb, J. A., Wetterlow, L. H., and Ipsen, J., Jr., *New Engl. J. Med.*, 267, 687(1962).
(25) Haas, R., Hennessen, W., Mauler, R., and Haupt, H., Can. pat. 648,166.

Quantitative X-Ray Diffraction Analysis of Intact Tablets

By G. J. PAPARIELLO, H. LETTERMAN, and R. E. HUETTEMANN

An investigation into the possible use of X-ray diffraction techniques to analyze intact tablets was made. The analysis of various pharmaceutical compounds in tablet form by X-ray diffraction was considered. Of those tested, only in glutethimide tablets, where the percentage of active component weight in total formulation weight is high, was the analysis of the intact tablet feasible. Methods of improving the reproducibility of this intact tablet analysis were considered. A procedure which makes it possible to assay 10 individual glutethimide tablets in 25 minutes was developed.

THE DEVELOPMENT of rapid analysis of unit dose formulations is a problem which has only recently come into the forefront. In 1961, Head (1) investigated the possible use of solid state fluorescence for the analysis of individual intact tablets and demonstrated that fluorescence was not totally acceptable for this purpose. The author did, however, suggest that X-ray diffraction techniques might possibly be more useful.

Taking advantage of this suggestion, an investigation into quantitative X-ray diffraction was initiated in this laboratory with the view that it might be possible to develop this technique to assay individual intact tablets both rapidly and automatically. It was felt that this investigation could uncover the answers to the following questions: What adaptations and precautions are necessary to obtain acceptable reproducibility in the analysis of an intact tablet by X-ray diffraction techniques? What concentration of a drug within the normal tablet matrix can be detected by these techniques? What precision can be obtained and how rapid can an analysis be run on individual intact tablets?

The theory of quantitative X-ray diffraction analysis is authoritatively considered by Klug and Alexander (2), while a brief but lucid account has recently been given by Shell (3). Consequently, a treatment of the underlying theory will not be considered here. However, it must be noted that the attainment of high precision in intensity measurements of diffracted X-rays demands careful attention to a number of factors, even when one is dealing with a carefully packed powdered sample. These factors—the particle size of the crystallite, preferred orientation, etc.—were considered in this work but could not be dwelled upon because of the nature of the sample. It is understood that an attempt at doing quantitative X-ray diffraction work without careful precautionary sample preparation is unusual and may be viewed with distress by a purist.

The work that is reported here is essentially a two-part study. The first part consists of an X-ray examination of various representative tablet formulations to determine what tablets might be assayed by this technique. The second part is a report on the analysis of glutethi-

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mide[1] tablets N.F. by this technique and the factors influencing this method.

EXPERIMENTAL

X-Ray Equipment.—A General Electric XRD-5 diffraction spectrogoniometer was employed in this work. X-ray radiation was provided by a copper target high intensity X-ray tube operated at 50 KVP and 15, 20, or 31 ma. A 3° beam slit, along with a medium resolution soller slit and a 0.1° detector slit made up the slit system used for most of this work. A 4° beam axis to target angle and a time constant of 1.0 seconds were employed. A gas proportional counter (General Electric No. 6 tube) was used as the detection device, its output being graphically displayed on a Leeds and Northrup Speedomax recorder. The scanning speed of the goniometer used throughout this study was either 2 or 0.2° per minute.

Sample Holders.—Powdered samples were packed in the normal shallow Bakelite trays $1^1/_4 \times 1$ in. in size; intact tablet samples were mounted in a specially designed sample holder fabricated out of Bakelite. This holder is illustrated in Fig. 1.

Qualitative Pattern Examination.—Powdered samples were finely powdered, passed through a

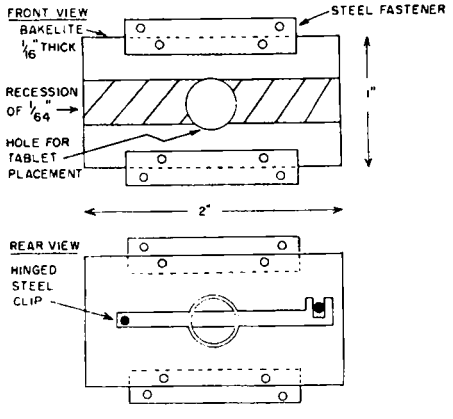


Fig. 1.—Intact tablet sample holder.

TABLE I.—TABLET FORMULATIONS INVESTIGATED

Tablet[a]	Active Component Wt. (%) in Total Formulation Wt.
Methylphenidate hydrochloride, 10 mg.	7
Hydrochlorothiazide U.S.P., 50 mg.	17
Tolazoline hydrochloride U.S.P., 25 mg.	17
Tripelennamine hydrochloride U.S.P., 50 mg.	25
Glutethimide N.F., 500 mg.	77

[a] Marketed as Ritalin, Esidrix, Priscoline, Pyribenzamine, and Doriden, respectively, by Ciba Pharmaceutical Co., Summit, N. J.

standard No. 320 sieve, and carefully packed in a shallow Bakelite tray. These samples were scanned from 5 to 30° 2θ. Intact tablets were merely placed in the specially designed intact tablet sample holder and scanned from 5 to 30° 2θ.

Quantitative Analysis of Glutethimide Tablets.—A tablet is placed in the intact tablet sample holder with its bisection facing forward. The tablet is oriented in the holder so that its bisection is perpendicular to the path of the X-ray beam. The sample holder is fastened in place on the diffractometer. A scan is made from 16 to 18° 2θ using a linear count range of 2000 counts per second and the instrumental conditions as previously indicated. This procedure is repeated for every sample tablet and the standard tablet.

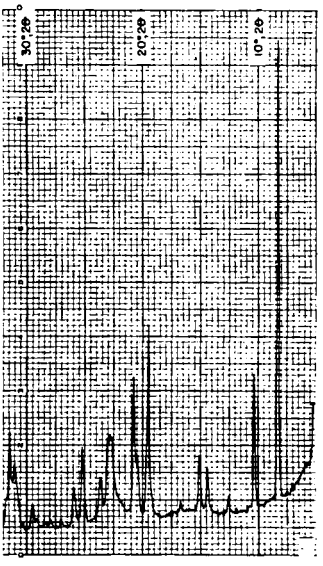


Fig. 2.—X-ray diffraction pattern of methylphenidate hydrochloride powder.

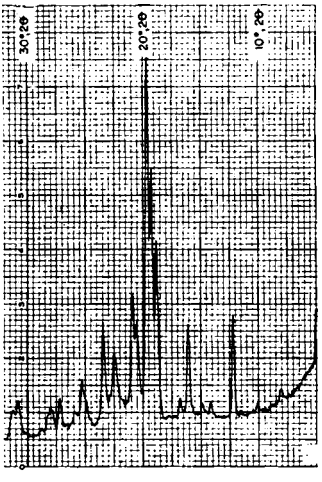


Fig. 3.—X-ray diffraction pattern of powdered methylphenidate hydrochloride tablet formulation.

For each tablet measure the height of the peak from its base is at approximately 17° 2θ. The equation (height of sample peak/height of standard peak)·100 = per cent glutethimide in tablet compares the peak intensity of the sample to that of the standard to obtain the analytical result.

RESULTS AND DISCUSSION

X-Ray Diffraction Examination of Representative Tablet Formulations.—The purpose of this part of the study was to determine what tablet formulations might possibly be analyzed by X-ray diffrac-

[1] Marketed as Doriden by Ciba Pharmaceutical Co., Summit, N. J.

tion techniques. From the outset it was understood that absorption and matrix interference preclude the use of this method for very small dosage tablet formulations. Nevertheless, there are many other tablet formulations where the percentage of drug weight in total formulation weight is at a level where analysis might be possible. Tablet formulations whose percentage of drug weight in total formulation weight was from 7 to 77% were considered. The commercial tablet formulations investigated are listed in Table I.

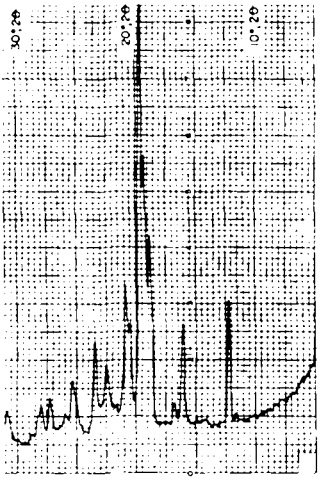


Fig. 4.—X-ray diffraction pattern of powdered methylphenidate hydrochloride placebo tablet formulation.

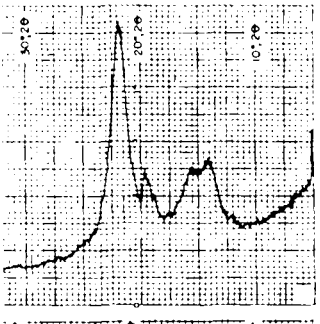


Fig. 5.—X-ray diffraction pattern of an intact methylphenidate hydrochloride tablet.

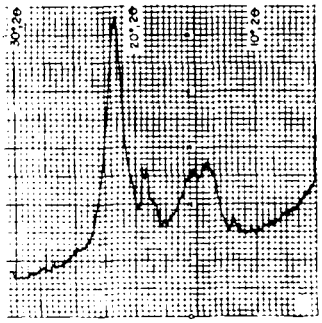


Fig. 6.—X-ray diffraction pattern of an intact methylphenidate hydrochloride placebo tablet.

The manner in which these various formulations were examined is quite simple. First a diffraction pattern of the powdered drug itself is obtained, and the most intense diffraction peaks noted. Then a diffraction pattern of the powdered tablet formulation containing the drug in question is taken. This pattern is studied, and one seeks out those diffraction peaks which were previously noted to be large on the pattern of the pure drug. A diffraction pattern of the powdered placebo formulation will aid in this examination, since the other components in a formulation have diffraction patterns of their own and can obscure or interfere with that of the drug itself. The final step is obtaining and examining diffraction patterns of the intact tablet and the intact placebo tablet.

Those patterns obtained for the methylphenidate hydrochloride investigation are shown in Figs. 2 through 6 as an illustration of the type of results obtained. By examining Figs. 2, 3, and 4, one will notice that a good many of the strong diffraction peaks seen in the methylphenidate hydrochloride powder pattern are obscured or absorbed in the pattern of the powdered formulation. However, if one were interested in performing the analysis on the powder rather than the intact tablet, it would be entirely possible since there are still measurable peaks at 8.1°, 14.3°, and 14.9° 2θ in the powdered formulation diffraction pattern. Figures 5 and 6 clearly demonstrate that the number and intensity of the diffraction peaks obtained from the dense intact tablet sample definitely diminishes. It can be seen that there are no diffraction peaks present which might reflect the amount of methylphenidate hydrochloride present in the intact tablet. All the intact tablet patterns of the compounds studied except for glutethimide were like those of methylphenidate hydrochloride and were determined to be unsuitable for use in an analytical method.

Diffraction patterns for powdered samples of glutethimide itself, glutethimide tablet formulation, and glutethimide placebo formulation indicated that most of the diffraction peaks were due to the glutethimide itself (see Figs. 7, 8, and 9). Seventy-seven

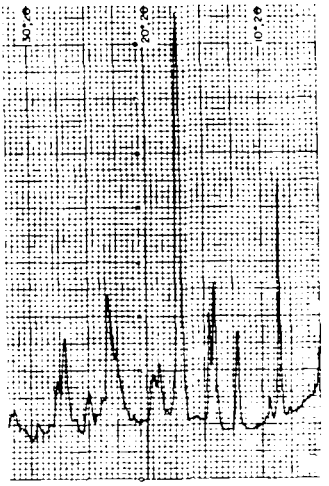


Fig. 7.—X-ray diffraction pattern of glutethimide powder.

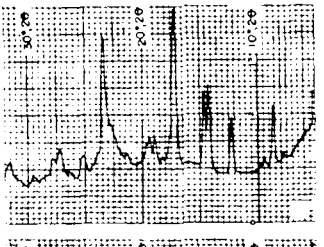


Fig. 8.—X-ray diffraction pattern of powdered glutethimide tablet formulation.

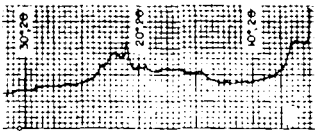


Fig. 9.—X-ray diffraction pattern of powdered glutethimide placebo tablet.

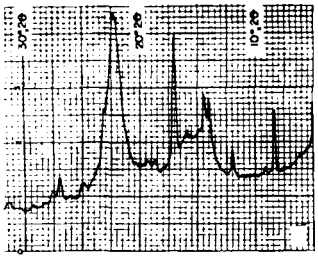


Fig. 10.—X-ray diffraction pattern of an intact glutethimide tablet.

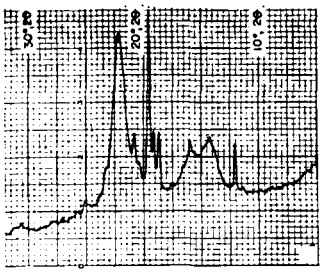


Fig. 11.—X-ray diffraction pattern of an intact glutethimide placebo tablet.

per cent of the tablet formulation is glutethimide, making it the predominant crystalline component in the formulation. Thus these diffraction results were expected. From an examination of Figs. 10 and 11 it can be seen that, although decreased, the glutethimide peaks in the intact tablet pattern are still distinguishable. The most intense diffraction peak, that at 17° 2θ, was chosen as the peak to use in an attempt to analyze glutethimide intact tablets quantitatively.

Quantitative Analysis of Glutethimide Tablets.—Having found a diffraction peak which was intense enough to warrant investigation in the case of glutethimide, it was then necessary to show that this peak did indeed reveal the quantity of glutethimide present in an intact tablet. Proof of this nature was obtained by preparing tablets of known composition and analyzing them by the diffraction technique. That is, several small batches of tablet formulation were prepared, and tablets were manually compressed using a Stokes model E tablet press.[1] Measurements were made on these tablets with ultraviolet spectrophotometry and X-ray diffraction techniques. A plot of the results of these analyses indicate that the intensity of the peak at 17° 2θ for the intact tablet is dependent on the quantity of glutethimide present in the tablet. Figure 12 shows that over a rather large concentration range this dependence is linear.

It was originally intended to use the tablets prepared by the above indicated method as standards for the analysis of the glutethimide production tablets. When this was attempted, however, it was noted that the diffraction intensities for the production tablets were less than that for the standard tablets. This observation was investigated and was shown to be a pressure effect. The pressure used in compressing the production tablets is, of course, much greater than that used in preparing the standard tablets. This pressure effect might be due to preferred orientation or a reduction in the size of the crystallites. It has been reported that the presence of a large proportion of crystals less than 0.2 μ in size in a sample will result in sufficient line broadening to produce an abnormally low peak height (4).

The existence of this pressure effect made it impossible to use the specially prepared standard tablets. It was necessary to use a production tablet as a standard tablet. Such a tablet was obtained by measuring the peak intensity at 17° 2θ for a group of production tablets. Each time a set of two tablets having identical peak height intensities was found—one member of the set was assayed by ultraviolet spectrophotometry, while the other was saved intact to be used as a standard.

[1] The authors are grateful to Dr. Arge Drubulis, who prepared these special tablets.

Table II compares the ultraviolet spectrophotometric and the diffraction analyses of 10 glutethimide tablets. The results for the diffraction analysis are given in peak height units (millimeters) and can be converted to milligrams per tablet by use of a standard production tablet. However, this conversion is not necessary if one is checking for dosage uniformity since limits in millimeters can be specified. The analysis of the intact tablets by diffraction took 25 minutes, whereas the ultraviolet spectrophotometric analysis of the powder of these tablets took over 2 hours. The reproducibility obtainable by the spectrophotometric method is greater than that obtained by the diffraction technique, but the diffraction technique's reproducibility is certainly acceptable if one were performing dosage uniformity analysis.

The monogram and scoring of a tablet have an effect on the absorption and scattering of the X-ray radiation. Consequently, one must always present the same surface similarly positioned in the X-ray beam if any sort of reproducibility is hoped for. Even when this precaution is taken, the reproducibility of measurements when the tablet is taken from the sample holder and then replaced in a similar manner is only ±2.5%. These results, however, are not greatly different from those obtained by Klug for the quantitative X-ray diffraction determination of powdered samples (5).

Shifting of the analytical peak from 16.93° to 17.18° 2θ has been noted. This shifting is the reason for obtaining a recorded scan and measuring the peak height rather than obtaining a count for a preset time at exactly 17.00° 2θ. Such peak shifting is not unusual when one is using large scanning speeds and large time constant values.

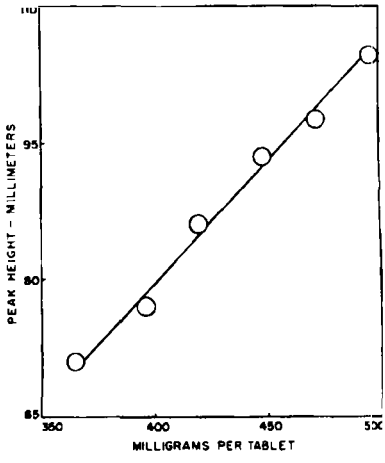


Fig. 12.—Relationship between the milligrams of glutethimide present in the intact tablet and the measured peak height intensity at 17° 2θ.

TABLE II.—COMPARISON OF DIFFRACTION AND ULTRAVIOLET SPECTROPHOTOMETRIC ANALYSES OF INDIVIDUAL GLUTETHIMIDE TABLETS

Tablet No.	Peak Ht., mm.	Deviation from Mean, mm.	Deviation from Mean, %	U.V. Analysis mg./Tablet	Deviation from Mean, mg.	Deviation from Mean, %
1	128	1	0.5	498	3	0.6
2	130	1	0.5	491	4	0.6
3	131	2	1.8	489	6	1.1
4	133	4	2.7	511	16	3.3
5	132	3	1.9	497	2	0.6
6	136	7	4.8	496	1	0.2
7	120	9	6.6	484	11	2.1
8	133	4	2.8	493	2	0.2
9	119	10	7.1	491	4	0.7
10	129	0	0.3	495	0	0.1
Av.	129	4	2.9	495	5	1.0

SUMMARY

This investigation has demonstrated that the percentage of drug weight in the total formulation weight must be large before one can consider the use of X-ray diffraction intact tablet analysis. From the data collected, it appears as if the drug must be at least 50% of the total formulation weight before analysis by this diffraction procedure could be contemplated. Of course, this judgement will depend on the crystalline character of the drug and the absorption effects of the tablet matrix.

It has also been shown that intact glutethimide tablets can be assayed by this X-ray diffraction technique with a reproducibility of ±3%.

REFERENCES

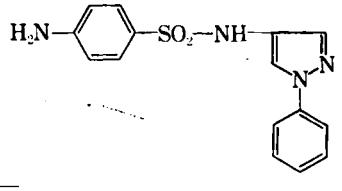
(1) Head, W. F., THIS JOURNAL, 50, 1041(1961).
(2) Klug, H. P., and Alexander, L. E., "X-Ray Diffraction Procedures," John Wiley and Sons, Inc., New York, N. Y 1954.
(3) Shell, J. W., THIS JOURNAL, 52, 24(1963).
(4) Christ, C. L., Barnes, R. B., and Williams ,E. F. ,*Ana Chem.*, 20, 789(1948).
(5) Klug, H. P., *ibid.*, 25 ,704(1953).

Drug Standards

Qualitative and Quantitative Tests for Sulfaphenazole

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation.

N' - (1 - PHENYLPYRAZOLYL - 5)SULFANILAMIDE; $C_{15}H_{14}N_4O_2S$; mol. wt. 314.37. The structural formula of sulfaphenazole may be represented



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Accepted for publication January 31, 1964.
Physicians Products Co., Inc., Petersburg, Va., has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.

Physical Properties.—Sulfaphenazole occurs as a white to cream-colored, fine crystalline powder, m.p. 178–182°, U.S.P. XVI Class I. It is freely soluble in acetone, sparingly soluble in alcohol, and practically insoluble in water. Sulfaphenazole dissolves in dilute mineral acids and in solutions of alkali hydroxides.

Identity Tests.—To about 100 mg. of sulfaphenazole, add 5 ml. of diluted hydrochloric acid, and boil gently for about 5 minutes. Cool in an ice bath, add 4 ml. of a solution of sodium nitrite (1 in 100), dilute to 10 ml. with water, and place the mixture in an ice bath for 10 minutes. To 5 ml. of the cooled mixture, add a solution of 50 mg. of betanaphthol in 2 ml. of sodium hydroxide solution (1 in 10): an orange-red precipitate is formed.