DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(2), 289-302 (1987)

VARIABILITY OF ASSAY RESULTS FOR HYDRALAZINE HYDROCHLORIDE TABLETS DUE TO THE USE OF ELECTRIC MILL COMMINUTION DURING SAMPLE PREPARATION

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INTRODUCTION

During the routine assay of a sample of film-coated hydralazine hydrochloride tablets by the method of USP XX (1), it was found that these tablets were significantly subpotent. As a verification, a second set of tablets from the same commercial lot was identically assayed by a second analyst, who found the potency to comply with the compendial requirements.

In view of the contrasting results, we decided to investigate the source of the discrepancy by comparing the experimental conditions used in both assays. In the former analysis the sample composite had been prepared in an electric mill, while in the latter

analysis a mortar and pestle were used instead. The USP XX does not specify the exact method of sample preparation.

The purpose of this investigation was to ascertain the role played by comminution of hydralazine hydrochloride tablets with an electric mill as a determinant of variability in the assay results, particularly since a review of the literature revealed that comminution is a potential source of false results (2-8).

EXPERIMENTAL

Apparatus - Tekmar Model A-10 analytical laboratory mill (Arthur H. Thomas Co., Philadelphia, PA 19105), with metal grinding bowl, blades, and cover; U.S. standard sieve No. 60 (Newark Wire Cloth Co., Newark, NJ 07104).

Samples - Commercial 25 mg hydralazine hydrochloride tablets from ten manufacturers (identified as A-J) were obtained from local sources. These samples included uncoated (manufacturers A-E), plain coated (manufacturers F-H), film coated (manufacturer I), and dry coated (manufacturer J) tablets. Additionally, tablets of other strengths (10, 50, and 100 mg) were selected from two of the manufacturers (H and J).

Assay Methods - Tablets were assayed for potency using an ultraviolet spectrophotometric method based on the conversion of hydralazine to a phthalazine compound by nitrite ions (9). Otherwise, assays were carried out using the official titrimetric method (1).

Compositing Methods - (A) Mechanical grinding plus manual grinding: A set of 20 randomly selected tablets was placed in an electric mill, and intermittently ground to a fine powder for periods of 25 sec. The powder bed was transferred to a glass mortar with the aid of gently tapping, making sure that the powder adhering to the walls of the grinding bowl, blade, and cover remained undisturbed. The powder bed was further ground with a glass mortar and pestle, and the powder was set aside pending assay (sample A-1). The powder that had adhered to the walls of the grinding bowl blade, and cover of the electric mill were carefully removed, mixed thoroughly, and set aside pending assay (sample A-2).

(B) Mechanical grinding plus manual grinding and sieving: A set of 20 randomly selected tablets was composited as described under (A), except that the powder bed was further ground with the aid of a glass mortar and

pestle to a powder that passed completely through a No. 60 sieve. The powder from the bed portion (sample B-1) was kept separately from a mixture of the powders obtained from the walls of the grinding bowl, blade, and cover of the electric mill (sample B-2). (C) Manual grinding: A set of 20 randomly selected tablets was ground with a glass mortar and pestle to a powder that passed completely through a No. 60 sieve (sample C-1). (D) Mechanical grinding plus direct dissolution: A set of 20 randomly selected tablets was made into a ground composite as in (A). The powder bed and the powder adhering to the metallic surfaces of the electric mill were quantitatively transferred with the aid of about 150 ml of water to a 250 ml volumetric flask. The various parts of the electric mill were rinsed with water, and the rinsings were added to the volumetric flask. The suspension was shaken, diluted with water to volume, and mixed. The solution was filtered through Whatman No. 1 filter paper, and the filtrate was assayed for potency (sample D-1). (E) Direct dissolution: Six randomly selected tablets were placed in a 500 ml iodine flask, mixed with 100 ml of water, and shaken mechanically for 15 min. Then the USP XX assay method for hydralazine hydrochloride

tablets was followed, starting with "Add 40 ml of hydrochloric acid".

RESULTS AND DISCUSSION

This study consisted of three phases: In phase I, the effects of three compositing methods, i.e., mechanical grinding, manual grinding, and direct dissolution, on the assay results of hydralazine hydrochloride tablets were examined. As shown in Table 1, a close agreement existed between the assay results for sample composites prepared by manual grinding and those prepared by direct dissolution. In contrast, both sets of results differed by about 4% of declared from those obtained after grinding the tablets with an electric mill. This difference is significant since the potency limits for hydralazine hydrochloride tablets set by USP XX (1) range from 95.0 to 105.0% of declared. In phase II, the drug content of powdered samples removed from the walls of the grinding bowl blade, and cover were compared with those obtained from the powder bed. For this purpose, uncoated and film coated tablets from three manufacturers (F, D, and I) were made into powdered composites by manual grinding, mechanical grinding, and mechanical grinding plus direct dissolution. The

Table 1. Assay results for 25 mg film coated hydralazine hydrochloride tablets (manufacturer F) powdered composites prepared by three compositing methods.

Compositing method	Found, % of declared[a]		
	Composite 1	Composite 2	Average
Mechanical Grinding	92.5	92.8	92.6
Manual grinding	96.4	95.5	96.0
Direct dissolution	97.0	96.5	96.8

[a]Assays were carried out by the official titrimetric method.

Table 2. Assay results for 25 mg hydralazine hydrochloride tablets powdered composites prepared by various compositing methods.

Manufacturer	Tablet type	Found, % of declared[a,b,c]			
		A-1	A-2	B-1	D-1
D	film-coated	79.1	320.4	96.4	97.5
F	film-coated	83.2	376.3	96.4	97.6
I	uncoated	77.4	314.6	97.5	98.1

[a] A-1: mechanical grinding, powder bed; A-2, mechanical grinding, metallic surfaces of the mill; B-1, manual grinding; D-1, mechanical grinding plus direct dissolution.

[b] Results for samples A-1 and B-1 are the average of duplicate assays. All other results were from single assays.

[c] All assays were carried out using a spectrophotometric method.

Table 3. Assay results for 25 mg hydralazine hydrochloride tablets powdered composites prepared using an electric laboratory mill.

Manufacturer	Composite No.	Found, % of declared[a]			
		Bed, run 1	Bed, run 2	Bed, average	Metallic surfaces[b]
A	1	85.2	85.5	85.4	266.3
	2	84.6	84.6	84.6	276.4
B	1	95.3	96.6	95.9	189.7
	2	94.3	94.0	94.1	218.9
C	1	97.6	98.1	97.8	126.7
	2	97.9	96.8	97.3	137.0
D	1	79.0	79.2	79.1	320.4
	2	76.4	77.9	77.1	342.8
E	1	88.3	86.3	87.9	250.7
	2	86.5	86.5	86.5	258.6
F	1	87.2	87.8	87.5	361.3
	2	88.3	89.1	88.7	351.0
G	1	81.0	79.8	80.4	172.8
	2	83.6	82.4	83.0	176.2
H	1	89.6	89.0	89.3	195.0
	2	88.9	89.0	88.9	207.7
I	1	77.2	77.6	77.4	314.6
	2	75.4	76.1	75.7	283.0
J	1	101.0	101.4	101.2	157.7
	2	102.1	101.1	101.6	158.6

[a] Assay results were obtained using a spectrophotometric method.

[b] All assay results are from single runs except for manufacturers D and I, which are reported as the average of two runs.

lower assay results noted for the manually ground composites are probably due to the segregation of the active ingredient, which became more concentrated on the walls of the glass mortar and pestle, and on the metallic sieve. In phase III, the use of a sieve was eliminated. However, the use of a mortar and pestle was retained to permit the reduction of the drug in the bed portion to smaller fragments. Accordingly, sample composites of 25 mg (manufacturers A-J) and 10, 50 and 100 mg (manufacturers C and D) hydralazine hydrochloride tablets were prepared using an electric mill. Assay of these powdered composites showed that, irrespective of their strength, their assay results (Tables 4 and 5) followed a pattern similar to that observed in phase II of the study, namely that higher values are given by powdered samples collected from the metallic surfaces of the mill than from the bed portion.

Greco (7,8) has examined the segregation of active ingredients from tablet matrixes following comminution. According to this author, segregation occurs mainly because of differences in particle size between the active drug and the tablet excipients, so as to cause a random and non-reproducible pattern of assay results for comminuted material obtained from

Table 4. Assay results for different strengths of hydralazine hydrochloride tablets (manufacturer C) powdered composites prepared using an electric laboratory mill.

Amount declared, mg/tablet	Composite No.	Found, % of declared: Bed, run 1	Bed, run 2	Bed average	Metallic surfaces
10	1	104.2	102.9	103.5	114.2
	2	103.5	103.2	103.3	124.2
25	1	97.6	98.1	97.8	126.7
	2	97.9	96.8	97.3	137.0
50	1	99.7	98.5	99.1	112.1
	2	98.5	99.0	98.7	114.7
100	1	95.1	95.2	95.2	137.2
	2	98.5	96.3	97.4	135.2

Table 5. Assay results for different strengths of hydralazine hydrochloride tablets (manufacturer D) powdered composites prepared using an electric laboratory mill.

Amount declared, mg/tablet	Composite No.	Found, % of declared: Bed, run 1	Bed, run 2	Bed, average	Metallic surfaces
10	1	81.1	82.9	82.0	402.7
	2	82.7	83.8	83.2	414.8
25	1	79.0	79.2	79.1	320.4
	2	76.4	77.9	77.1	342.8
50	1	79.2	79.7	79.4	457.9
	2	81.7	81.9	81.8	471.6

different portions of the compositing vessel. In contrast, the segregation pattern observed in the present study for powders deposited on the metallic surfaces of an electric mill is non-random and reproducible. The present results also indicate that the active ingredient is uniformly distributed throughout the bed portion, a finding that cannot be explained in terms of differences in particle sizes alone. A plausible explanation is to recognize that ordered units are formed within the powdered bed during the grinding process, and that most of the powder adhering to the walls of the grinding bowl, blade, and cover of the mill arrange into an ordered mixture. Ordered units are combinations of particles of different substances which become attached to surfaces in a fixed proportion upon particle interactions (10). An ordered mixture is a mixture composed of ordered units (11).

In this study, an electric mill was used for comminuting tablets to powdered composites. During the grinding process, the blade is considered to move in a horizontal plane of rotation and to cause the tablet materials to impact against the metallic surfaces of the mill (12). Upon impaction, the tablet will break apart at compression faults to yield frag-

ments of different sizes and/or compositions (13, 14). Particles consisting of single tablet constituents, whether an active ingredient or an excipient, are termed primary particles (14). Primary particles are free to interact with each other. In the state of primary particles, hydralazine hydrochloride is likely to form ordered units throughout the powdered mixture with some of the tablet excipients. These solid-solid interactions are accentuated by the electrostatic energy generated by the moving blade upon impaction with the primary particles and fragments (15), causing the ordered units to become selectively retained by the metallic surfaces of the mill.

Since hydralazine hydrochloride becomes concentrated in the ordered units found on the metallic surfaces, the assay values for this type of sample is expected to be higher than those obtained for samples collected from the powder bed. The environment within the bed portion is more complex than in the ordered mixtures deposited on the surfaces of the mill. In addition to the ordered units, the bed portion also contains tablet fragments and free-flowing primary particles. Further rotation of the blade will cause the formation of additional ordered units within the bed which, when retained by the

metallic surfaces of the mill, will further lower the assay values of the bed portion.

Discrepancies in the assay values for 25 mg hydralazine hydrochloride tablets from manufacturers A-J (Table 1) are probably due to differences in product formulation, a known contributing factor to the formation of different ordered units (16, 17).

CONCLUSIONS

The results of this study suggest a possible explanation to account for the discrepancy in assay values noted between samples obtained from the powder bed of an electric mill and those obtained from the surroundings. During grinding with an electric mill, the active drug will become incorporated into ordered units, a large number of which become attached to the metallic surfaces of the mill, thus lowering the concentration of drug in the bed portion. If this situation is overlooked, quantitative transfer of a representaive portion of the tablet powdered composite will be impossible and the analytical results will be unreliable. The routine use of manual grinding of tablets with a mortar and pestle will lead to acceptable assay results since the number of ordered units retained by the glass surfaces is minimal.

Optimally, the sequential use of grinding with an electric mill and direct dissolution of the combined powders from the various parts of the electric mill in a suitable solvent, will negate the effect of surface interactions and render the assay values valid.

ACKNOWLEDGMENT

The author wishes to thank Cesar A. Lau-Cam, Ph.D., Science Advisor, Food and Drug Administration, New York Regional Laboratory, and Professor of Pharmaceutical Sciences, St. John's University, College of Pharmacy and Allied Health Professions, Jamaica, New York, for his invaluable assistance in the preparation of this paper. Thanks are also extended to Barry Mopper and Deborah Genova, Food and Drug Administration, New York Regional Laboratory, for their excellent technical help in various parts of the study.

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