Attachment 2

MEMORANDUM

To: Laboratory Director, NYK (HFR-2160)
Laboratory Director, ATL (HFR-460)
Laboratory Director, DET (HFR-5360)
Laboratory Director, MIN (HFR-5460)
Laboratory Director, DAL (HFR-6160)
Laboratory Director, KAN (HFR-7160)
Laboratory Director, LOS (HFR-9260)
Laboratory Director, SEA (HFR-0160)
Laboratory Director, BLT (HFR-3260)
Director, WEAC (HFR-1300)
Chief, Biopharmaceutics Laboratory Branch (HFD-524)

From: Don C. Cox, Chemist, Methods Research Branch,
National Center for Drug Analysis (HFH-300)
Date: May 28, 1980
Subject: Dissolution Collaborative Study, Prednisone Tablets by Apparatus 2 of the USP,
National Center for Drug Analysis, April 1980

We are grateful for your participation in this study. The following materials are being sent under separate cover:

1. Instructions to collaborators
2. A reprint of "Guidelines for Dissolution Testing"
3. 500 mg of prednisone standard material
4. 100 tablets of NCDA Performance Standard No. 2, with instruction sheet containing acceptance limits
5. Four bottles, identified A through D, each containing 24 tablets

The Division of Biometrics has agreed to examine the results of this study. After examining the results of the intralaboratory study recently conducted at NCDA, they observed a trend which correlated with the particular day on which the results were obtained. The trend is not dramatic, but the Division of Biometrics feels it would be most desirable if all the laboratories followed the same test schedule. By doing so, the results from all laboratories would be subjected to the same trend.

A test schedule is given in Table 1. We have indicated that two weeks should be allowed to complete the study. Though most laboratories can easily complete the study in less than two weeks, one weekend will interrupt the sequential collection of data. The essential point of the test schedule is to start with Samples A and B on a Friday and to follow with Samples C and D on Monday.

We realize some laboratories may not be able to participate in the collaborative study if this schedule must be followed; the schedule may be impossible because of a heavy workload or because of problems with the results from the Performance Standard. If such situations exist, please ignore the test schedule and complete the study in a manner which is most expeditious for

you. If more than one run is required to obtain satisfactory results with the Performance Standard initially, please report the results from all runs, as indicated in the instructions, and indicate which set of data you feel is the "correct" or final set.

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Table 1. Test Schedule for Dissolution Collaborative Study	
Weekday	**Activity**
Monday	Review instructions and references
Tuesday	Set up and/or check out apparatus
Wednesday	Trial(s) with Performance Standard
Thursday	Trial(s) with Performance Standard
Friday	Run six tablets each from Samples A and B
Monday	Run six tablets each from Samples C and D
Tuesday	Run six tablets each from Samples A and B
Wednesday	Run six tablets each from Samples C and D
Thursday	Run six tablets from Performance Standard
Friday	Write-up of study

NCDA DISSOLUTION TEST
PERFORMANCE STANDARD #2,
Prednisone Tablets, 10 mg

Procedure

Determine the quantity of prednisone dissolved at 30 min, expressed as per cent of the labeled amount. Use Apparatus 2, as described in the USP, with the plastic dissolution vessels from Elanco Products Division and rotate the paddle at 50 rpm. Use 500 ml freshly deaerated water as the Dissolution Medium.

Measure the amount of prednisone in solution in filtered portions of the dissolution medium at the wavelength of maximum absorbance at about 242 nm in comparison with a solution of known concentration of USP reference standard. An amount of alcohol not to exceed l% of the total volume of standard solution may be used to bring the prednisone standard into solution prior to dilution with dissolution medium. Perform the test on six individual tablets.

Acceptance Limits:	%
Individual tablets	30 - 50
Average of six tablets	35 - 43

Note: Under normal conditions the tablets disintegrate quickly into coarse, insoluble granules, most of which stay on the bottom of the vessel in the form of a cone throughout the test. If excess gases are present in the medium, most of the tablet material will be dispersed throughout or on the surface of the medium. High results (in the range of 70 to 80% of declared) will be obtained.

The tablets are sensitive to the alignment parameters of the apparatus (levelness of the base, verticality of shafts, and centering of vessels). High results will be obtained if the apparatus is misaligned. Generally speaking, the better the alignment is for an apparatus, the closer the average result of six tablets will be to 35%.

This performance standard was packaged and labeled expressly for FDA use. It has no legal status and is not to be used in lieu of any calibrator issued by the USP.

THE BOTTLES SHOULD BE KEPT TIGHTLY CAPPED AND PROTECTED FROM EXTREME HEAT AND UNUSUAL HUMIDITY CONDITIONS.

Dissolution Collaborative Study Prednisone Tablets by Apparatus 2 of the USP National Center for Drug Analysis, April 1980

Samples Provided
NCDA Performance Standard No. 2 (100 tablets) and four samples of prednisone tablets, each sample consisting of 24 tablets, are provided. A total of 12 tablets will be tested from each of the five samples in the following sequence, six tablets being tested at a time: Performance Standard, A, B, C, D, A, B, C, D, Performance Standard.

One report sheet is attached to these instructions. Please make photocopies of this report sheet, as needed. One sheet will be required to report the dissolution results of each six tablets. Use the back of the report sheets to record such observations as vessel weights, standard weight, and comments.

Background Information
Review the provided reprint "Guidelines for Dissolution Testing" from Pharmaceutical Technology, the slide-tape presentation "Guidelines for Dissolution Testing, July 1978," the operating manual for your dissolution apparatus, and the section covering dissolution in the Fifth Supplement of USP XIX. This information will help you interpret the following instructions. In some instances the instructions will differ from the information you will find in the references cited above. Please follow the instructions given in this collaborative study carefully, even when they differ from the official USP instructions.

Equipment
A. Model 72S or Model 72R six-spindle dissolution drive mounted on Easi-Lift stand. Hanson Research Corp., P. 0. Box 35, Northridge CA 91324.

B. Fluorocarbon-coated, stainless-steel, center-mounted paddles, 3/8-inch diameter, 40 cm long. Hanson Research Corp., No. 65-700-030.

C. Plastic dissolution vessels. Elanco Products Division, No. EQ1900, Eli Lilly and Co., P. 0. Box 1750, Indianapolis, IN 46206.

D. Acrylic vessel covers, without guide bushings, that are six mm thick and have a 10-mm slot cut from the center hole to the edge of the cover. Hanson Research Corp., No. 72-110.

E. Transparent water bath. If one is not on hand, a 20-gal. glass aquarium, 12 x 12 x 30 inches, can be purchased locally.

F. Circulator-heater device, capable of maintaining the water bath at a constant ($\pm$ 0.2 ^{o}) temperature near 37 ^{o} (see instructions below) and maintaining a smooth, constant circulation of water.

G. Glass syringes, 50 ml, with Luer-Lok tips

H. Glass aliquoting tubes, ca 4-mm O. D. These tubes are attached to the tips of the syringes by means of rubber tubing and are used in drawing dissolution medium from the dissolution vessel. Two sets of six tubes are required, one set for 500-ml volumes and one set for 900-ml volumes.

For 500-ml volumes: Cut five tubes to lengths of 88 mm $\pm$ 2 mm. Cut one tube to a length of ca 140 mm. Using a flame, bend this longer tube sharply at a 45 ^{o} angle from its cylindrical axis at a point which is 107 mm from one end of the tube.

For 900-ml volumes: Cut five tubes to lengths of 63 mm $\pm$ 2 mm. Cut one tube to a length of ca 130 mm. Bend this longer tube, as directed above, at a point which is 82 mm from one end.

I. Membrane filters, 0.8-um porosity, 2.5-cm diameter. Millipore Corp., No. AAWP, or equivalent.

J. Swinnex-type filter holders, 2.5 cm. These holders must lock onto the 50-ml syringes (Item G above).

K. 900-ml and 500-ml volumetric flasks marked T.D./T.C., Kimble Products, P. 0. Box 230, Vineland, NJ 08360, Nos. 28045-900 and 28045-500, respectively. These flasks were designed for use in dissolution testing. Though convenient, they may be replaced by 1-liter graduated cylinders, calibrated to contain 903 ml $\pm$ 0.5 percent at 37 ^{o}, and 500 ml volumetric flasks marked T.C. only.

L. Water bath capable of holding six 900-ml volumetric flasks and maintaining a temperature of 37 ^{o} -37.5 ^{o}.

M. A 2.5-cm depth gauge and a centering tool, both available from Winchester Engineering and Analytical Center. Contact Mr. Albert A. Serino, Supervisor, Facilities Service Section, WEAC/HFR-1330. FTS 839-8700.

This centering tool was designed by Mr. Serino for dissolution testing. The tool consists of two flat plastic disks, one smaller than the other, that are cemented together so that they have a common center. A 3/8-inch hole passes through the center, and a 3/8-inch slot extends from the hole to the edge of the larger disk. The larger disk is beveled so that it will fit part way down into the top of the dissolution vessel or into a hole in the base of the dissolution apparatus. The smaller disk has a fitted aluminum band, which can be removed, and a setscrew embedded along the radius of the disk. Neither the aluminum band nor the setscrew will be used in this study.

N. An 8-inch or 9-inch torpedo level with two bubble indicators at right angles to each other. Obtainable from any local hardware store.

O. Ruler graduated in millimeters.

P. Thermometer graduated in 0.1 ^{o} increments with a range which includes 37 ^{o}.

Q. Stop watch graduated in 0.2-second increments with a range of 30 minutes or more.

Equipment- Setup

A. Set the apparatus on the empty waterbath. The base of the Easi-Lift stand must be in a horizontal plane. Place the torpedo level on the base to check that the base is level from side to side and from front to back. If the base is not level, it must be shimmed. Care must be taken in the shimming process. If the base does not have adequate support, e.g., if only the corners of the base are supported, it may warp with time or the base may not be stable.

B. Make sure that the uprights and cross members of the tubular framework which support the six-spindle drive are rigid. Use standard allen wrenches to tighten the framework if necessary.

C. Insert the paddle shafts upside down through the top of the spindles of the six-spindle dissolution drive. The manufacturer cautions not to force the paddle shafts through the spindle past the fluorocarbon coating on the paddle shaft. We have found the paddle shafts go all the way through the spindles to the blades with no difficulty. This observation may not hold for you. Set the three lower collars (one around each upright) at a distance of 7 cm from the base of the stand. Make the measurement from the top of the collar to the base. Bring the drive support assembly down until it rests on all three collars and set the three upper collars to hold the support assembly in place. (It may be necessary to lift the counterweight slightly in order to bring the back of the support assembly to rest on the back collar.) Place the WEAC centering tool sequentially in each hole in the base, position the corresponding paddle shaft until it is just above the centering tool, and check to see how well the shafts are centered with the holes. The shafts should be centered within an estimated 1 mm. If all the shafts are off in the same direction, raise the dissolution drive, loosen the adjustment slide screws underneath the drive, and reposition the drive until all shafts are centered within 1 mm. If the shafts deviate in different directions, for example, if the shafts are not parallel, use your best judgment to position all the shafts in their "best fit" within 1 mm of the center of the respective hole. If there is no adjustment that will bring all shafts within 1 mm of the center of the respective hole, the equipment is not suitable for use in this study. Tighten the adjustment slide screws to hold the dissolution drive securely.

D. Bring the drive-support assembly back down on all three collars and lock in place. Push the paddle shafts down through the spindles as far as they will go without use of excessive force. Check the shafts for verticality by placing the torpedo level against the shafts. Each shaft should be checked from two directions: viewing the shaft from the side and from the front. Necessary adjustment is easily accomplished from front to back by means of the thumbscrew control. Adjustment from side to side is more difficult. In most cases it is possible to loosen the tube clamps which hold the adjustment slides and move the clamps up or down slightly for side-to-side adjustment. In a few cases it may be necessary to prop up one side of the drive. A short steel rod placed in a right-angle laboratory clamp can be attached to one cross member of the drive support and used to prop up the low side so that the shafts are vertical.

An alternative procedure is to remove the front bolt from the adjustment slide on the low side and insert a flat washer between the slide and the base of the drive head. Replace the bolt and tighten.

When you are certain that the shafts are vertical, check the centering of the shafts a second time. It is possible that the centering procedure and verticality procedure may have to be repeated more than once in order to bring the apparatus into proper alignment. Keep in mind that a compromise "best fit" alignment may be necessary if the shafts are not parallel.

Adjusting the drive is exacting and sometimes frustrating work. It is critical that these operations be performed as well as possible, however. When they are satisfactorily completed and all parts of the apparatus are rigidly held, it will not be necessary to repeat them for the remainder of the study.

E. Number the vessels one through six using a stylus or china marker. The three holes in the back of the base, as you face the apparatus, are defined to be positions one, two, and three from left to right. The three holes in the front of the base are defined to be positions four, five, and six from left to right. Place vessel one in position one, vessel two in position two, etc. This numbering system will be used throughout the study. For example, report the dissolution results from vessel one in position one as result number one for each sample.

With the paddles still inverted in the spindles, lock the drive support in position on the collars and center each vessel around the shaft using the WEAC centering tool. If there are locator lugs on your apparatus, use them to lock the vessels in place. If you have an older Easi-Lift stand not equipped with the lugs or other means (aluminum “fingers,” for example) of locking the vessel in place, trace the outline of each flange of each vessel onto the base when the vessel is being held around the shaft with the centering tool. Exercise care in seeing that the vessel is relocated exactly each time it is put back into position. Care should be taken not to force an alignment between the vessel and the shaft. It is possible to flex the shaft in the spindle. If the vessel is pushed against the side of the hole, it can appear that the vessel is centered around the shaft; but when the tool is removea, the shaft will spring back if it has been under stress.

F. Bring the drive support up, take out the inverted paddles, and insert them into the spindles in their normal position. Bring the drive support back down to rest on the collars, and set the paddle depth 2.5 cm from the bottom of the vessels using the depth gauge. Tighten each chuck to hold the paddle at the correct depth.

G. Mount the circulator-heater externally to the bath. The only direct contact that the circulator-heater and mounting support may have with the bath is where the circulator-heater enters the bath liquid. Transfer 500 ml of water to each vessel. Fill the bath until the water level matches the water level in the vessels. Lower the drive support to the working position and rotate the paddles at 50 rpm. Cover the vessels with the cover plates and adjust the bath temperature until the liquid in each vessel is maintained at $37^{o} \pm 0.5^{o}$. The bath temperature must usually be 38^{o} to 38.5^{o} to keep the liquid in the vessels at 37^{o}.

It is convenient to add 15 g to 30 g of copper acetate to the bath followed by ca 50 ml of acetic acid to retard mold. It is also convenient, when the vessels are removed, to mark the water-bath level with a china marker. In this way the correct water level can be maintained from day to day without putting in the vessels. The water level must be maintained at two different levels, one for

500-ml volumes and one for 900-ml volumes in the vessels. The bath level must be adjusted accordingly for these volumes when they are changed from one to the other.
H. Set up a second water bath capable of holding six 900-ml volumetric flasks and of maintaining a temperature of 37 ^{o} to 37.5 ^{o}. This bath is used to preheat the dissolution medium to the correct temperature.

Reagent and Standard Preparation
A. Dissolution Medium: Purified water, USP, which has been sufficiently deaerated. Distilled or deionized water which passes the USP specifications for purified water may be used. Even if the water is distilled on the day it is used, it may not be adequately deaerated. Deionized water is not sufficiently deaerated without further treatment.

Purified water may be brought to a boil for a few minutes and then cooled and used within 24 hours. Purified water under pressure (e.g., water from a deionizer) may be sprayed into a container which is under vacuum. A suitable spray nozzle is available under the trade name "Fogg-It" from the Fogg-It Nozzle Co., P. 0. Box 16053, San Francisco, CA 94116. Both of these procedures are demonstrated in the slide-tape presentation "Guidelines for Dissolution Testing, July 1978." Other means of deaeration of purified water are stirring the water by means of a magnetic stirrer while the water is under vacuum or by use of ultrasonic energy. Deaerated water at 25 ^{o} must not be agitated excessively. It must be used within 24 hours. A sufficient volume must be prepared at one time for analysis of six tablets and for dilution of the standard material used in the analysis.

B. Standard Preparation: Dry the provided prednisone standard material for 3 hours at 105 ^{o}. Accurately weigh about 80 mg and transfer to a 50-ml volumetric flask. Partially fill the flask with 95 percent ethanol and shake until dissolved. Ultrasonic energy will aid in dissolving the material. Dilute to volume with 95 percent ethanol and mix. This solution becomes the stock standard solution. It is stable for at least 1 week.

For 5-mg tablets: Transfer 5.00 ml of stock standard solution to a 1-liter volumetric flask and dilute to volume with dissolution medium from the same batch used to perform the dissolution test on the tablets. Do not use after 24 hours.

For 10-mg and 50-mg tablets: Transfer 5.00 ml of stock standard solution to a 500-ml volumetric flask and dilute to volume with dissolution medium from the same batch used to perform the dissolution test on the tablets. Do not use after 24 hours.

Preheating Dissolution Medium
Fill the 500-ml or 900-ml volumetric flasks, marked T.D./T.C., to the T. D. mark with dissolution medium at room temperature. Place them in the water bath at 37 ^{o} to 37.5 ^{o} until they have equilibrated, usually in 20 minutes.

If these flasks are not available, 500-ml volumetric flasks, marked T. C. only, may be used to measure the 500-ml volumes. A 1000-ml graduated cylinder may be used to measure the 900-ml volumes. The dissolution medium is preheated in other containers (e.g., gallon reagent bottles)

and transferred to the graduated cylinder at 37 ^{o} to a measured volume of 903 ml $\pm$ 0.5 percent. This volume is then transferred to the vessels.

The Dissolution Test

Transfer the preheated dissolution medium to each vessel. Lower the dissolution drive and lock it in place. Place the cover plates over the vessels so that the slots in the plates are located behind the shafts in positions one, two, and three and in front of the shafts for positions four, five, and six. Start paddle rotation. After 15 minutes, check the temperature in vessel five. It should be 37 ^{o} $\pm$ 0.5 ^{o}. Check the paddle rotation. It should be 50 revolutions per 60 seconds. If the Model 72R is used, adjust the speed to give 50 revolutions in not less than 60 seconds and not more than 61 seconds.

Hold tablet one over the slot of the cover plate of vessel one not more than 1 cm from the vessel wall. Drop the tablet into the vessel with the paddle rotating. Immediately start the stopwatch. (These instructions differ from those given in the USP).

From a similar position drop tablet two into vessel two after 1 minute, accurately timed. Likewise drop tablets three through six into their respective vessels at accurately timed, 1-minute intervals thereafter.

Attach the set of longer glass tubes to the syringes with short pieces of rubber tubing when using 500 ml of dissolution medium. Attach the set of shorter glass tubes when using 900 ml of dissolution medium. Use the syringe with the bent tube exclusively with position two of the apparatus. Attach the tubes so they are reasonably flush with the syringe tips and in line with the cylindrical axis of the syringes.

At exactly 30 minutes on the stop watch insert the glass tube of the syringe through the slot of the cover plate of vessel one at a point midway between the vessel wall and the paddle shaft. Lower the syringe vertically until the syringe shoulders rest on the cover plate and draw 50 ml of dissolution medium into the syringe. Remove the glass tube, connect the filter holder to the syringe tip, discard the first 25 ml of filtrate, and collect the remaining 25 ml in a suitable container.

At exactly 31 minutes insert the bent glass tube of the syringe through the slot of the cover plate of vessel two at a point midway between the vessel wall and the paddle shaft and lower the tube vertically until the bend in the tube is even with the cover plate. Draw 50 ml of dissolution medium into the syringe, remove the glass tube, and filter, discarding the first 25 ml of filtrate.

Draw dissolution medium from vessels three through six in the same manner as used for vessel one at accurately timed, 1-minute intervals thereafter.

Record on the report sheet the temperature inside vessel five at the start of the test and after the test has been completed. If the Model 72R is used, record on the report sheet the seconds (to the nearest 0.2 second) the drive takes to make 50 revolutions after the test has been completed.

Procedures

Weigh each of the dry, empty plastic dissolution vessels to the nearest 0.1 g. Record these weights on the back of the first report sheet (the report sheet used to record the dissolution results of NCDA Performance Standard No. 2).

NCDA Performance Standard No. 2: Prednisone Tablets, 10 mg

Use 500-ml volumes preheated to 37 ^{o}. Operate the apparatus as instructed under The Dissolution Test. Using the dissolution medium as the reference, record the absorbances, measured at 242 nm in a 1-cm cell, on the report sheet and calculate the percent of labeled amount dissolved as described under Calculations. (If the dissolution medium is used to "zero" the spectrophotometer at 242 nm, read the net absorbance directly and ignore the left absorbance column on the report sheet.)

Check the test results against the acceptance limits given in the instruction sheet provided with the performance standard. If the obtained results fall outside of these limits, contact: Don Cox, FTS 279-4135, before continuing.

If the results from six tablets fall within the acceptance limits, continue with the other samples, testing six tablets at a time, in the following sequence: A, B, C, D, A, B, C, D, Performance Standard. Result one is associated with position one, result two with position two, etc., in all cases.

Sample A. Prednisone Tablets, 5 mg.

Use 500-ml volumes preheated to 37 ^{o}. Operate the apparatus as instructed under The Dissolution Test. If a spectrophotometer which has expanded-scale capability e.g., 0 to 0.5 absorbance full scale) is available, the expanded scale may be used if desired. Using the dissolution medium as the reference, record the absorbances, measured at 242 mm in a 1-cm cell, on the report sheet and calculate the percent of labeled amount dissolved as described under Calculations.

Sample B. Prednisone Tablets, 5 mg.

Proceed as directed under Sample A.

Sample C. Prednisone Tablets, 50 mg.

Use 900-ml volumes preheated to 37^{o}. Adjust the water level in the bath. Operate the apparatus as instructed under The Dissolution Test. Accurately dilute 10 ml of filtrate to 25 ml with disso ution medium. Using the dissolution medium as the reference, record the absorbances, measured at 242 nm in a 1-cm cell, on the report sheet and calculate the percent of labeled amount dissolved as described under Calculations.

Sample D. Prednisone Tablets, 50 mg.

Proceed as directed under Sample C.

Calculations

Use the following format for calculating and reporting results on the report sheet.

For 5- and 10-mg tablets:

Factor 1 = (A mg/50 ml) · (5 ml/B ml) · 500 ml · (100/C mg)

For 50 mg tablets:
Factor 1 = (A mg/50 ml) · (5 ml/500 ml) · (25 ml/10 ml) · 900 ml · (100/50 mg)

where A is the weight of standard, in mg per 50 ml of 95 percent ethanol; B is the final volume, in ml, of diluted standard solution; and C is the amount of drug, in mg, declared per tablet.

For 5-, 10-, and 50-mg tablets:

Factor 2 = (Factor 1)/(net standard absorbance)

Percent of Labeled Amount Dissolved = Factor 2 · (net sample absorbance)

REPORT SHEET

Date of Test:
Chemist:
Laboratory:
Sample:
Means of measuring volumes:

Temperature of medium in vessel five at:

Start of Test End of Test

Model 72R used Model 72S used

sec./ 50 rev. at end of test.

Factor

Factor

	Abs.	Net Abs.	Percent of Labeled Amount dissolved
Stnd			
Blk		0.000	
1			
2			
3			
4			
5			
6			
Avg.			
S.D			