International Journal of Pharmaceutics, 16 (1983) 97–103
Elsevier

Standardization of in vitro dissolution assays

G. Mazuel *, E.M. Cohen ** , M. Bastide ***

* *Merck Sharp and Dohme-Chibret Research Laboratories, 63203 Riom (France);* ** *Merck Sharp and Dohme Research Laboratories, West Point, PA 19486 (U.S.A.); and* *** *Faculté de Pharmacie, 63000 Clermont-Ferrand (France)*

(Received December 28th, 1982)
(Accepted January 26th, 1983)

Summary

A previous communication by Konieczny et al. (1980) has high-lighted the advantages of determining the dissolution rate of both a test sample and a control, or reference lot of the formulation, at the same time in the same equipment. One can immediately reject aberrant test sample values or normalize the test sample results by comparison with the results obtained for the control dosage units. This publication demonstrates that for the dosage form tested herein the standardization technique could adequately compensate for small changes in test conditions which influence dissolution, such as slight changes in pH and temperature of dissolution media.

Introduction

Unlike many other assays performed in product quality control, dissolution testing measures a dynamic variable which reflects a non-equilibrium condition. For this reason, and because of the nature of the test, the results obtained are greatly influenced by mechanical, chemical and physical parameters associated with either the apparatus itself or its environment. A great deal of work has been done in order to identify the interfering parameters and minimize their effects on the dissolution process (Cartensen et al., 1978; Cartwright, 1979; Cox et al., 1979; Hanson, 1975, 1977, 1979; Embil and Torosian, 1979; Underwood and Cadwaller, 1976). The parameters cited include vibrations, shaft wobble, shaft tilt, shaft centering, positioning of the basket or of the paddle, positioning of the sampling probe, control of temperature, de-aeration of dissolution medium, and variation in speed of agitation.

In order to ensure that the units in the field operate in a somewhat similar fashion, calibrators were made available, and a suitability test incorporated into the USP XX (1980). This test is performed on prednisone disintegrating tablets and salicylic acid non-disintegrating tablets. The test is performed according to the USP procedure (provided along with the calibrators). If the results fall outside the acceptance range, the operating parameters such as those listed above should be re-adjusted, the ultimate step being the replacement of the dissolution apparatus.

Despite all the precautions, it has already been reported that two units of the same design, validated according to the USP suitability test, and located in two different laboratories yielded significantly different results (Konieczny et al., 1980).

This raises several comments. (1) The use of calibrators is not without problems; Hanson and Hanson (1979) and Taborsky-Urdinola et al. (1981) reported that storage conditions affect the calibrators dissolution rate; therefore, validation checks could possibly be performed with non-satisfactory calibrators.

(2) The acceptance range may still be too broad; two units giving calibration values near the extremes of the acceptance range may give different results on test samples. (3) Validation runs are only performed periodically: perhaps monthly or even less frequently. As a consequence, if one obtains an 'out of specs' validation run, the data obtained since the last acceptable validation could be considered as being suspect. The validation runs reflect the instrument performances at the time they are being performed, but give little information on performances between checks, or variations due to the operator. Since the instrument performances can be easily altered by its environment this may be an important point. (4) In order to monitor the instrument performances on a continuous basis, calibrators should be run at the same time as samples, but this would not be practical since the calibrators are only valid for one set of dissolution conditions, which may vary from the desired test conditions. (5) The parameters affecting the sample dissolution rate (mechanical, physical or chemical) may not affect samples and calibrators to the same extent. This of course does not question the utility of the calibrators, which are necessary to establish that all the dissolution units in the field operate similarly, at least on these two reference tablets. Nonetheless, different formulations may react differently to changes in the dissolution parameters, e.g. pH, temperature, vibration.

We are very much in the situation of a classical assay, and analytical chemists have long solved the problem in other techniques, by carrying through the entire analytical procedure and at the same time as the samples, a standard of the compound to be assayed whose response will be used in the calculations. The use of the standard corrects small operating or instrumental variations, or catches the operator's attention in cases of an abnormal response. Using a standard during each assay, however, does not replace the periodic instrument calibrations which must be performed in order to ensure that the equipment performances are satisfactory.

The concept of using a standard for in vitro dissolution testing has been reported (Konieczny et al., 1980). The authors showed that using a standard (a 'reference batch' of the product tested) successfully corrects important variations imposed on the system (speed of rotation varied from 75 rpm normally used, down to 25 rpm, and up to 100 rpm). The dissolution rate determination is performed on 4 samples

and 2 standards run simultaneously. The work being reported in the present publication is a follow up to these studies.

Materials and Methods

Study samples — indomethacin 75 mg controlled-release capsules [1].

Dissolution medium — pH 6.2 phosphate buffer 0.05 M. All materials used were standard reagent grade.

Dissolution equipment — 6 station, Hanson 72 SL.

Dissolution unit [2] equipped with rotating paddles (USP XX, Apparatus 2).

Assay method — indomethacin content of samples taken from the dissolution experiment were assayed by UV spectrophotometry at the standard wavelength of 320 nm. Calibration curves were linear in the concentration range of interest.

Results and Discussion

The data collected are presented in Table 1. Column 1 shows the average dissolution values (n = 4) obtained under purposely modified conditions (temperature and/or pH) on 2 lots of the product. These figures are not corrected and therefore would be the values reported for the lot. Column 4 shows the average dissolution values (n = 4) obtained under the normal assay conditions on the same samples. Analysis of variance (Snedecor and Cochran, 1957) performed on the individual dissolution values (not shown in Table 1), indicates that the data in column 1 are statistically different ($P \leqslant 0.05$) from the corresponding data in column 4. The average dissolution data obtained under normal conditions on the reference standard (selected arbitrarily for the purpose of the demonstration) are shown below Table 1. These figures are the average of values obtained during 5 independent determinations. Column 2 shows the average dissolution value (n = 2) obtained on the standards run at the same time as the samples. The corrected values are obtained on each individual dissolution value, their average being reported in column 3. The correction is performed using the following equation:

$$V = \frac{SM \times A}{B}$$

where V = corrected value, SM = % dissolved in sample, A = % dissolved in standard, and B = established % dissolved for standard.

Analysis of variance (Snedecor and Cochran, 1957) performed on the individual values (not shown in Table 1) indicates that the data in column 3 are not statistically

[1] Chrono-Indocid (MSD-Chibret, Paris).

[2] Hanson Research, Northridge, CA.

TABLE 1

SUMMARY OF THE AVERAGE IN VITRO DISSOLUTION DATA OBTAINED ON A SUSTAINED-RELEASE PRODUCT UNDER NORMAL AND MODIFIED CONDITIONS, WITH AND WITHOUT STANDARD CORRECTION (STANDARD DEVIATION OF VALUES IS GIVEN IN PARENTHESES)

Sample and test conditions	Sampling time (h)	Average dissolution values not corrected (n = 4)		% Dissolved average dissolution of the standard (n = 2)	Average dissolution values corrected (n = 4)	Average dissolution values obtained under normal test conditions (n = 4)	
		Column 1		Column 2	Column 3	Column 4	
Lot no. 1, pH 6.2, 36°C	1	13.6	(0.6)	14.7	11.0	11.8	(2.0)
	2	21.7	(0.4)	23.3	18.8	19.1	(1.6)
	3	29.0	(0.6)	30.3	25.6	24.8	(1.3)
	4	34.1	(0.7)	35.5	30.5	29.5	(2.2)
	6	42.2	(0.9)	43.7	37.9	40.0	(2.5)
	7	45.3	(1.0)	47.2	41.0	40.4	(2.0)
Lot no. 1, pH 6.3, 37°C	1	17.0	(0.9)	16.7	12.1	11.8	(2.0)
	2	26.6	(0.8)	27.5	19.6	19.1	(1.6)
	3	35.2	(1.0)	36.0	26.2	24.8	(1.3)
	4	40.8	(1.1)	41.8	31.0	29.5	(2.2)
	6	50.0	(1.5)	50.9	38.6	40.0	(2.5)
	7	53.0	(1.5)	53.8	42.0	40.0	(2.0)
Lot no. 2, pH 6.2, 38°C	1	14.7	(0.8)	15.7	11.1	11.2	(2.1)
	2	24.7	(0.3)	27.2	18.4	19.4	(3.2)
	3	32.4	(1.4)	34.7	25.0	26.0	(3.4)
	4	38.1	(1.7)	40.4	30.0	30.6	(3.6)
	6	46.9	(1.3)	49.4	37.3	38.5	(3.9)
	7	50.2	(2.0)	52.4	40.9	41.2	(4.1)
Lot no. 2, pH 6.1, 36°C	1	10.6	(0.7)	10.8	11.7	11.2	(2.1)
	2	17.4	(0.8)	18.2	19.4	19.4	(3.2)
	3	23.3	(0.8)	24.0	26.0	26.0	(3.4)
	4	27.7	(1.1)	28.5	30.9	30.6	(3.6)
	6	35.0	(1.0)	35.8	38.3	38.5	(3.9)
	7	37.8	(0.8)	38.4	42.0	41.2	(4.1)

In vitro dissolution of the reference obtained under normal test conditions (average of 5 different runs).

1 H	2 H	3 H	4 H	6 H	7 H

different ($P \leqslant 0.05$) from the corresponding data in column 4. In the absence of a standard, the instrument performances can be verified by monitoring the dissolution medium temperature and pH, the rotating speed, the vibration level, or any other variable that one may think will affect the sample dissolution rate. However, if one or more of these parameters fall outside the specifications for only part of the dissolution run, it will be very difficult to correlate the variations to an absolute effect on the sample. The purpose of the standardization is to correct the effect of these variations, or simply assess the importance of their effect. Since sample and standard are likely to be affected to the same extent, detailed knowledge of all interfering parameters is not essential.

As previously noted, Konieczny et al. (1980) showed that the use of a standard can successfully compensate for important variations in the basket rotating speed. In the present study, we imposed on the system small variations of pH and temperature such as those likely to be encountered during normal operations. The data obtained showed that these variations, which have a significant effect on the assay results, are successfully compensated by the standardization.

As was done in the previous publication, the standard dissolution data were used to obtain a correction factor for the calculation of the sample corrected value. One may object to this procedure, and rather may prefer to use the standard dissolution data as a simple check on instrument performances during a particular run. The standard data base, to which two values are added after each dissolution run, allows the determination of the assay baseline variation. If for a specific run, the standard values are outside the expected variation limits, the data obtained should be considered as being suspect and eventually rejected statistically.

The dissolution standard of a particular formulation will be selected, as outlined in the referenced publication, much in the same way an analytical standard is selected. The lot should be selected for its acceptable in vivo properties, or because it compares favorably in vitro to a lot which has acceptable in vivo properties. It should also have a very good dissolution uniformity. The lot should be stored under selected conditions to ensure stability, and it should be retested periodically. The established dissolution values for the lot will be obtained after several dissolution runs are performed possibly in several laboratories. Within one laboratory, several runs performed on the standard will give an estimation of the daily variations of the instrument performances. In addition, each time a dissolution run is performed, two values are obtained for the standard which can be added to the standard data base. These two values will provide a constant monitoring of the standard dissolution rate, and will allow its replacement if they drift below the acceptable level.

Since most commercial dissolution apparatuses are constituted of 6 stations, we used 2 stations for the standard and 4 for the sample. The standardization technique may be better performed on equipment with more dissolution stations to handle more samples and possibly more standards.

Conclusion

An earlier publication showed that two different units of the same design, validated according to the USP procedure will not necessarily give the same dissolution values on the same lot of a given product. This may be attributed to the fact that the mechanical, physical and chemical parameters which determine the dissolution rate may not influence to the same extent the calibrators, and the samples studied. Also the calibrations are performed periodically and do not reflect the equipment performances during a sample test. The use of a standard, which is a preselected lot of the same formulation as the one tested, and run at the same time, allows correction of the effect of large variations in stirring speed and also small variations in pH and temperature. The standard can be used directly to calculate a corrected value for the sample, or simply as a check on the dissolution run. In the latter case, if the dissolution values are outside the expected range of variation the data obtained can be rejected. This technique offers the advantage of monitoring the equipment performances during every dissolution test and over its entire cycle. This is particularly important for tests that run several hours unattended.

The use of the standards should complement the use of calibrators but not replace them. the calibrators being used as the universal references. The validation checks using the USP calibrators can be compared to instrument calibration operations performed in other analytical techniques where a standard is nevertheless carried through the procedure along with the samples to be assayed.

The ability of standardization to correct variations in operating conditions, has been clearly established. This technique should be very helpful especially when it comes to comparing results obtained on the same product in different laboratories. It may possibly find applications in the comparison of data obtained on different equipment designs.

References

Cartensen, J.T., Lai, T.Y.F. and Prasad, V.K., USP dissolution IV. Comparison of methods. J. Pharm. Sci., 67 (1978) 1303–1307.

Cox, D.C., Douglas, C.C., Furman, W.B., Kirchhoeffer, R.D., Myrick, J.W. and Wells, C.E., Guidelines for dissolution testing. Pharm. Tech. Int., Jan. (1979) 37–49.

Cartwright, A.C., Practical aspects of dissolution testing. Drug Devel. Ind. Pharm., 5 (1979) 277–291.

Embil, K. and Torosian, G., Effect of instrumental vibration levels on dissolution. J. Pharm. Sci., 68 (1979) 1336–1338.

Hanson, W.A., Effects of Vibrations on Dissolution Rates, Beckman Conference on Dissolution Techniques. Oct., 1975.

Hanson, W.A., Solving the puzzle of random variables in dissolution testing. Pharm. Tech. Int., Oct. 1977.

Hanson, W.A., Investigation on the Effect of Input Variables on the Results of Dissolution Tests, IPT/PAC, Acad. Pharm. Sci., San Francisco, CA, Oct., 1979.

Hanson, W.A. and Hanson, R.A., Effect of moisture on the dissolution rate of prednisone tablets. Pharm. Tech. Int., March, 1979.

Konieczny, J.M., Cohen, E.M., Thomas, A. and Van Dame, H.C., Validation technique of dissolution test for control of drug quality and bioequivalence — instrumentation and equipment, Interphex, Sept. 1980. Pharm. Tech. Int., 88–94.

Snedecor, G.W. and Cochran, W.G., Statistical Methods, 6th Edn., Iowa State University Press, 1957.
Taborsky-Urdinola, C.J., Gray, V.A. and Grady, L.T., Effect of packaging and storage on the dissolution of model prednisone tablets. Am. J. Hosp. Pharm., 38 (1981) 1322–1329.
Underwood, F.L. and Cadwaller, D.E., Effects of various hydrodynamic conditions on dissolution rate determinations. J. Pharm. Sci., 65 (1976) 697–700.
U.S. Pharmacopoeia XX, 1980, National Formulary XV, U.S. Pharmacopoeia Convention, Rockville, MD.