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INTER-LABORATORY REPRODUCIBILITY OF RELEASE TESTS FOR SUPPOSITORIES

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ABSTRACT

Inter-laboratory reproducibility of representative release tests, namely paddle, Muranishi and dialysis tubing methods for suppositories were investigated using two kinds of suppositories of indomethacin, oily and water-soluble types. The inter-laboratory differences for the water-soluble suppository determined by paddle and modified Muranishi methods were small whereas the differences for the oily suppository determined by Muranishi and dialysis tubing methods were large. The release rate of oily suppository by Muranishi method was significantly reduced when the cylindrical cell containing suppository was placed slightly lower. The release rate by dialysis tubing method was decreased by addition of a small volume of test fluid into the dialysis tube. These variables probably contributed to the inter-laboratory differences for the oily suppository. Neither Muranishi or dialysis tubing method should be employed for quality control of oily suppositories unless their reproducibility is significantly improved.

INTRODUCTION

Bioavailabilities of suppositories are often greatly influenced by the release rate of drugs,[1-4] meaning that quality control of suppositories by release tests is required for the bioavailability assurance. However, no release tests have been officially established for suppositories, for which several factors should be taken into consideration including *in vitro/in vivo* correlations, reproducibility, handling

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of equipment's and cost. Among these factors, reproducibility is very important for quality control, which should be clarified before we use the release tests. But there have been few studies investigating the reproducibility, especially among different laboratories.

The present study was undertaken to clarify the inter-laboratory reproducibility of typical release tests for suppositories. Two model suppositories, an oily and water-soluble types of indomethacin, were employed in this study, which were prepared using witepsol and polyethylene glycol, respectively. For oily suppository, Muranishi[5] and dialysis tubing methods[4] shown in Fig. 1 were employed, because the former method has been extensively used in our country and the latter one has often provided significant *in vitro/in vivo* correlations.[1-4] For the water-soluble suppository, modified Muranishi and paddle methods were used.

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EXPERIMENTAL

Preparation Both of oily and water-soluble suppositories were prepared by the fusion method. An oily suppository contained 50 mg of indomethacin, 700 mg of witepsol W35 and 50 mg of witepsol E35, and a water-soluble suppository contained 50 mg of indomethacin and 1450 mg of polyethylene glycol 1540.

Release test The tests were carried out at 37.0±0.2 °C using pH 7.2 phosphate buffer employed for dissolution test of indomethacin capsules in Japanese Pharmacopoeia (JP). The amounts of indomethacin released in the test medium were determined spectrophotometrically and the average released amounts were determined after five release runs.

1) Oily suppository The release rates were determined by a dialysis tubing method in 900 ml of the test medium and by Muranishi method in 500 ml of the medium as previously reported.[4] In the dialysis tubing method[4] (Fig. 1), a dialysis tube (Union Carbide 18/32) was washed with distilled water and its one end was tied. The dialysis tubing was soaked in the test fluid and used after squeezing out the test fluid manually with fingers. A test suppository was put into the tubing and immersed in 900 ml of the test fluid with a 5 g lead weight so that the tied end of the dialysis tube was 8 cm below the surface of test fluid and 2 cm from the wall of the vessel. The paddle was rotated at 50 rpm at the position of 1.5 cm above the bottom of vessel. In Muranishi method[5] (Fig. 1), a TMS-103 apparatus (Toyama Sangyo Co. ltd., Osaka, Japan) was employed. A membrane filter (Millipore SSWP, pore size 3 μm) was fastened to the bottom of the cylindrical cell, which was immersed in the test fluid so that the bottom of the

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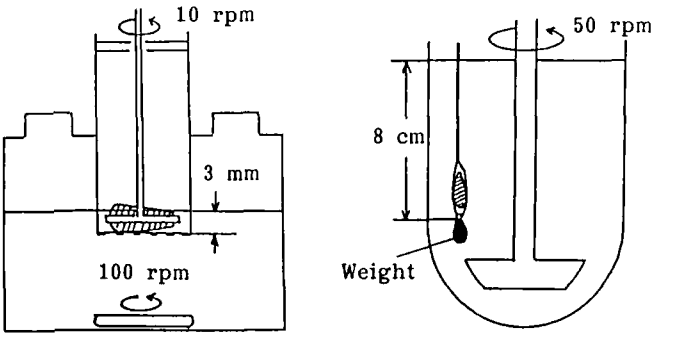

FIGURE 1.
Nuranishi (Left figure) and dialysis tubing methods (right figure)

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cell was 3 mm below the fluid surface. A test suppository was stirred with a steel rod at 10 rpm at the position of 2 mm above the bottom of cylindrical cell. The test medium in the outer vessel was agitated with a magnetic stirrer at 100 rpm.

2) Water-soluble suppository The release rates were determined in 900 ml of the test fluid by JPXII paddle method at 50 rpm and in 600 ml of medium by a modified Muranishi method which was agitated with a magnetic stirrer at 40 rpm. In modified Muranishi method, no membrane was used and suppositories were not stirred with the steel rod. The cylindrical cell was fixed so that the bottom of the cell was 10 mm below the medium surface.

Statistical test Analysis of variance was applied to detect the difference in release rate among laboratories. The intra- and inter-laboratory variabilities were assessed with coefficients of variation determined using following equations[6],

$$CVr\ (\%) = 100\ (MSr/M)$$

$$CVa\ (\%) = 100\ (MSa - MSr)/(N \cdot M)$$

where CVr : Coefficient of variation for intra-laboratory
CVa : Coefficient of variation for inter-laboratory
MSr : Residual mean square
MSa : Mean square for laboratories
M : Overall average value
N : number of measurements in each laboratories

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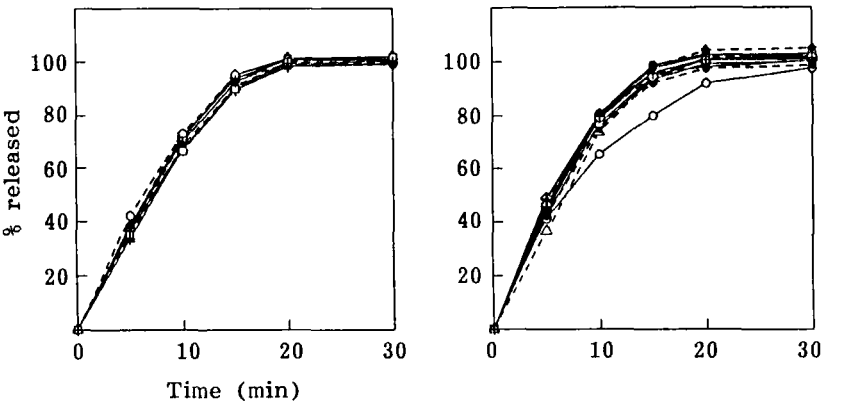

FIGURE 2.
Mean released amount–time curves of indomethacin from a water-soluble suppository determined by a modified Muranishi method in eleven laboratories (left Figure) and JPXII paddle method in nineteen laboratories (right figure).

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RESULTS AND DISCUSSION

Water–soluble suppository Fig. 2 shows the mean released amount–time curves of indomethacin from a water-soluble suppository determined in different laboratories by a modified Muranishi method and JPXII paddle method, respectively. The release profiles among laboratories were similar except for one determined by the paddle method. However, because of the small residual error of analysis of variance, statistically significant differences ($p<0.01$) were observed among laboratories in the released amount at each sampling time in both methods. Table 1 shows the maximum and minimum values in release rate among laboratories and coefficients of variation for intra- and inter-laboratories. The inter-laboratory coefficients of variation were much smaller compared with those for oily suppository shown in Table 2. The findings indicate that both of paddle and modified Muranishi methods will provide reproducible data for other water-soluble suppositories also.

Oily suppository Fig. 3 shows the mean released amount–time curves of indomethacin from a witepsol suppository in different laboratories determined by Muranishi and dialysis tubing methods. The release curves varied greatly among laboratories. Statistically significant differences ($p<0.01$) were observed among

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TABLE 1.

Minimum and Maximum Values Among Laboratories in Average Amount (%) of Indomethacin Released from a Water-soluble Suppository Determined by a Modified Muranishi Method in Eleven Laboratories and by the Paddle Method in Nineteen Laboratories and Coefficients of Variation for Intra- (CVr) and Inter-laboratories (CVa)

	Muranishi				Paddle			
Time	% released		CVr	CVa	% released		CVr	CVa
(min)	Min.	Max.	(%)	(%)	Min.	Max.	(%)	(%)
5	33.5[a)]	42.2	4.4	6.5	36.5	49.0	4.7	6.2
	(2.6)	(2.5)			(0.5)	(2.6)		
10	66.2	73.0	4.1	3.2	65.0	80.4	4.5	4.4
	(1.8)	(2.7)			(3.4)	(2.9)		
15	88.7	94.4	2.4	2.1	79.1	97.7	2.8	3.9
	(1.0)	(1.1)			(4.1)	(2.0)		
20	96.6	99.7	0.9	1.0	90.4	102.2	1.4	2.4
	(0.9)	(1.6)			(2.0)	(0.6)		

a) Figures in the parentheses show SD (n=5).

TABLE 2.

Minimum and Maximum Values Among Laboratories in Average Amount (%) of Indomethacin Released from a Water-soluble Suppository Determined by Muranishi Method in Seven Laboratories and by a Dialysis Tubing Method in Eleven Laboratories and Coefficients of Variation for Intra- (CVr) and Inter-laboratories (CVa)

	Muranishi				Dialysis Tubing			
Time	% released		CVr	CVa	% released		CVr	CVa
(hr)	Min.	Max.	(%)	(%)	Min.	Max.	(%)	(%)
1	8.6[a)]	18.7	23	54	2.5	11.0	24	69
	(2.2)	(4.8)			(0.5)	(1.2)		
2	15.7	30.2	18	53	4.5	20.2	18	66
	(4.9)	(4.2)			(0.9)	(1.9)		
3	21.1	42.6	17	53	7.4	28.3	16	64
	(6.6)	(7.5)			(1.3)	(1.9)		
4	25.5	54.5	20	54	9.6	34.6	15	62
	(7.8)	(6.3)			(1.7)	(1.9)		

a) Figures in the parentheses show SD (n=5).

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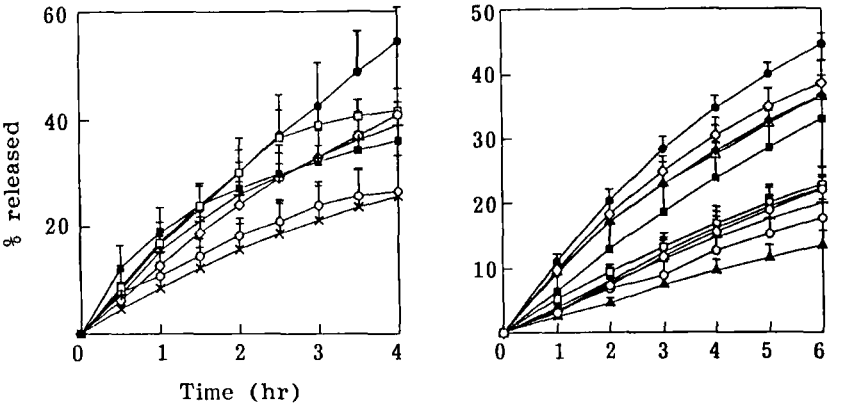

FIGURE 3.
Mean released amount–time curves of indomethacin from a oily suppository determined by Muranishi method in seven laboratories (left figure) and the dialysis tubing method in eleven laboratories (right figure). The vertical lines shows SD.

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laboratories in the released amount at each sampling time in both methods. Table 2 shows minimum and maximum released amounts among laboratories and the coefficients of variation for intra- and inter-laboratories. The inter-laboratory coefficients of variation were 53–54 % for Muranishi method and 62–69 % for dialysis tubing method, that were very large than those for water-soluble suppositories shown in Table 1.

In order to clarify the causes of the large inter-laboratory variabilities, effects of some testing variables on the release rate were investigated. The releasing rate in Muranishi method was hardly affected by the rotatory speed of steel rod in the range between 10 and 25 rpm (data not shown). The drug release was, however, drastically reduced when the cylindrical cell containing a suppository was lowered only 1 or 2 mm from the specified position (Fig. 4). By lowering the cell, the volume of test fluid in the cell increased and suppositories floated. In this circumstance, the frequency of suppositories to be hit by the rotating steel rod was significantly reduced and the disintegration of suppositories was delayed. This may be the main reason for the retarded releasing rate. The release rate was accelerated by elevating the testing temperature (Fig. 5) as expected from previous reports.[2,3] From these findings, we are able to deduce that position of the cylindrical cell is a major factor causing the large

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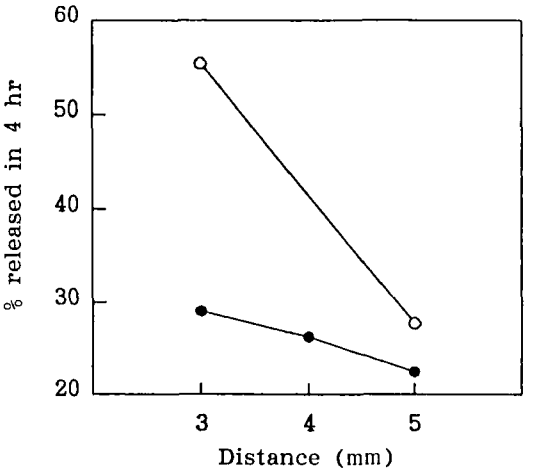

FIGURE 4.

Mean release rates (% released in 4 hr) of indomethacin from an oily suppository determined by Muranishi method in laboratories AO (○) and EO (●) at different distances between the bottom of cylindrical cell and the surface of test fluid.

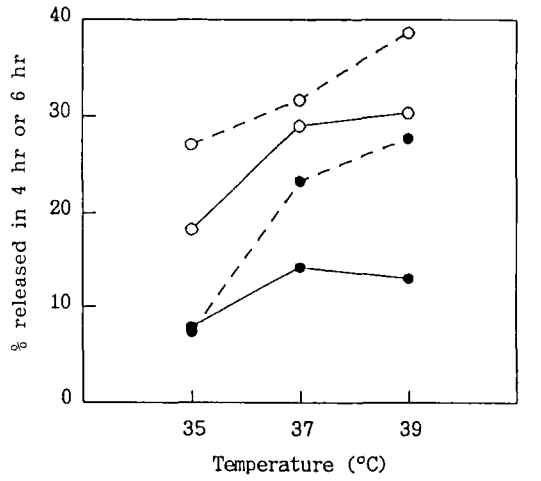

FIGURE 5.

Mean release rates of indomethacin from an oily suppository determined at different temperatures by Muranishi (○) and dialysis tubing methods (●) in laboratories EO (—) and HO (- - -). Release rates for Muranishi and dialysis tubing methods were shown as % released in 4 and 6 hrs, respectively.

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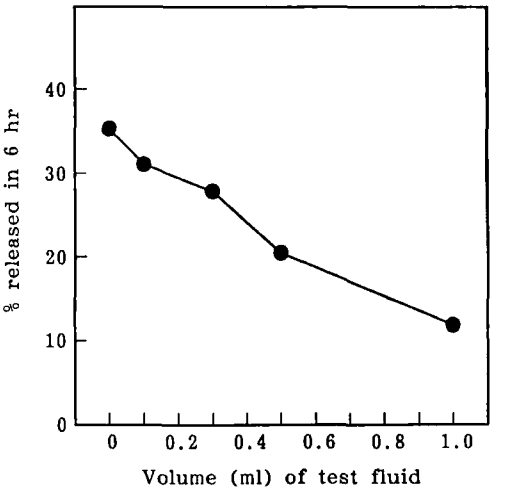

FIGURE 6.
Effects of the volume of test fluid added into the dialysis tube on mean release rates (% released in 6 hr) of indomethacin from an oily suppository.

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inter-laboratory variability in releasing rate, although other unknown factors may also contribute to the variability. The reason is because we have investigated the reproducibility without a great care about the cell position and it is not easy to set the cylindrical cell exactly at the specified position of 3 mm below the fluid surface (usually, 1 or 2 mm deviation from the specified level is unavoided). The testing temperature was not supposed to be the main reason for the large inter-laboratory variability as we paid much attention to the temperature control for oily suppositories.

In the dialysis tubing method, the release rate was hardly affected by the rotation speeds of the paddle in the range between 50–150 rpm (data not shown), but was influenced by the testing temperature (Fig. 5). However, the temperature would not be the major cause for the inter-laboratory difference in releasing rate as stated above. The drug release was significantly decreased by addition of a small volume of test fluid into the dialysis tube (Fig. 6). The retarding effect was enhanced with the increase of volume of the test fluid. In the presence of test fluid, the suppository did not spread well in the dialysis tube, which is probably the main reason for the delayed release. In this tubing method, dialysis tubes soaked in the test medium were used after squeezing out the medium manually with fingers. However, complete removal of the test fluid was very difficult, meaning

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that a small quantity of test medium would remain in the tubes and it probably varies depending on the squeezing skill of each investigator as previously reported by Yamazaki et al.[7] These findings indicate that the variability in the remaining amount of test fluid in dialysis tubes contributed to the inter-laboratory differences in releasing rate, although other unknown factors might also contribute to the differences.

Considering the difficulty of exact set of Muranishi's cell at the specified position and that of complete removal of test fluids remaining in dialysis tubes, it does not seem practical to use Muranishi and dialysis tubing methods for quality control of oily suppositories without any suitable improvement in apparatuses or testing methods.

From this study, we can conclude that both paddle and modified Muranishi methods may be used as a quality control test for water-soluble suppositories, but neither Muranishi or dialysis tubing method should be employed for oily suppositories unless their reproducibility is markedly improved.

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