Determination of Water Vapor Transmission Rate for High Density Polyethylene Screw-cap Bottles

Executive Summary

The Product Quality Research Institute (PQRI) Container Closure Working Group undertook testing using four different laboratory sites to find ways to improve USP General Chapter <671> Containers – Permeation. Water Vapor Transmission Rate (WVTR) testing was performed to construct weight gain profiles due to moisture ingress for 60 mL high-density polyethylene (HDPE) bottles with intact or broached induction seals when stored at 23°C and 40°C and 75% RH. The profiles were compared to determine the time needed to reach steady state, the time, duration and number of data points needed for WVTR calculation, the variability of the data due to sampling and lab to lab variation, and to determine if empty bottles (i.e. "controls") are needed for accurate WVTR determination. This report summarizes the data collected from the four testing laboratories –Abbott, Merck Sharp & Dhome, Pfizer, and sanofi-aventis.

The recommendations are as follows:

- USP Chapter <671> should be rewritten or replaced.
- For the purpose of moisture-barrier comparison, WVTR testing should be conducted at 40^{o}C/75% RH
- The test duration should be 35 days, and containers weighed at 7 day intervals
- WVTR should be calculated by regression analysis over the period from day 7 to day 35 (5 data points).
- Blanks (Controls) are not necessary for bottles with induction-sealed closures, and should not be included in the test design
- If calcium chloride is used as the desiccant, it should be pre-dried at 210^{o}C, rather than 110^{o}C as specified in USP <671>

Introduction and Objectives

The purpose of this study was to assess the opportunity to improve USP <671>. Weight gain profiles, due to moisture ingress, of closed 60 mL high density polyethylene (HDPE) screw-cap bottles (Multiple Unit Container Systems) were obtained during storage at 23^{o}C/75% RH and 40^{o}C/75% RH. The profiles were used to determine the time needed to reach steady state, the duration of the test and number of data points needed for calculation of water vapor transmission rate (WVTR), the variability in data due to samples and testing labs, and to determine if controls (blanks) are needed for accurate WVTR determination. The controls were bottles containing glass beads in place of desiccant. The origins of this study also lie, in part, in a previous publication[1] that

[1] Barry J., J. Bergum, Y. Chen., R. Chern , R.Hollander, D.Klein, H. Lockhart, D. Malinowski, R. McManus, C. Moreton, A. Mueller, L. Nottingham, C. Okeke, D. O'Reilly, K. Rinesmith and S. Shorts (PQRI Container-Closure Working Group), "Basis for Using Moisture Vapor Transmission Rate per Unit

provided a theoretical basis for using WVTR per unit dose as a means to compare different container-closure systems.

Materials and Methods

The bottles used were 60mL HDPE with 28mm double lead semi interrupted threads. The HDPE resin was Phillips Marlex 5502 BN. The bottles were manufactured by Rexam. The closures used were 28mm diameter polypropylene (PP) with Squeeze and Turn child-resistant feature. The PP resin was Phillips-Sumika HLN 120-01. The closures were manufactured by Rexam. The cap liners were supplied by Selig with FS 1-7 inner-seal and C25P liner. The materials of construction for the inner-seal and liner were 0.0015" medium density polyethylene (MDPE)/0.001" Al foil/Heat Tac Wax/0.005" Paper/0.030" Polyolefin Foam.

The bottles were uniquely identified, then filled with an appropriate amount of anhydrous calcium chloride desiccant and induction-sealed in accordance with USP <671>. See Appendix A for details about selection of desiccant. The containers were all filled and sealed at the same time at one site (Pfizer). For details of sample preparation, see the protocol in Appendix B. The seals of a designated number of these bottles were then broached and the bottles re-capped according to the procedure prescribed in USP <671>.

On receipt at the testing laboratory, the seals of a designated number of these bottles were then broached and the bottles re-capped according to the procedure prescribed in USP <671>. The broached seals were prepared as follows: The cap of the induction sealed bottle was removed, and the seal was cut around the inner circumference of the mouth with a sharp razor blade. The cut-out material was removed, taking care to leave a flat, unbroken area of inner-seal on the land of the bottle finish. The caps were then re-applied to the bottles in accordance with the procedure in USP <671>. The details of the broaching procedure are given in Appendix C.

Subsequently, both the intact and broached bottles were stored in environmental chambers controlled at 40±2°C/75±5%RH or 23±2°C/75±3%RH at the four sites: Abbott (AB), Merck Sharp & Dhome (MK), Pfizer (PF) and sanofi-aventis (SA).

A testing protocol was developed that required each laboratory to perform WVTR tests as follows:

1) 15 Broached bottles with desiccant, 10 control samples without desiccant stored at 40±2°C/75±5%RH and weighed at 7 day intervals for 49 days.

2) 15 Broached bottles with desiccant, 10 control samples without desiccant stored at 23±2°C/75±3%RH and weighed at 7 day intervals for 49 days.

Product in the Evaluation of Moisture-Barrier Equivalence of Primary Packages for Solid Oral Dosage Forms", Pharmacopeial Forum (2005) 31(1) Jan-Feb, 226-269

3) 15 Intact bottles with desiccant, 10 control samples without desiccant stored at 40±2°C/75±5%RH and weighed at 14 day intervals for 98 days.

4) 15 Intact bottles with desiccant, 10 control samples without desiccant stored at 23±2°C/75±3%RH and weighed at 14 day intervals for 98 days.

The weight gain of each sample was measured and recorded according to the procedure given in the protocol. The average rate of water vapor permeation (WVTR) in mg per day per bottle was calculated according to the protocol.

Results/Discussion

Weight Gain Profiles

Figures 1 and 2 show the mean weight gain (mg/bottle) profile (average of 15 bottles with desiccant, or 10 control bottles) for broached bottles over the testing period for each laboratory. As can be seen in the profile plots, there is no apparent induction period, and the weight gain profiles are linear over the testing interval. The broached bottles containing desiccant showed an increase in weight over time, with a higher rate for the higher temperature condition, which was expected. The controls show very little change over time.

Broached with Desiccant

Figure 1

40°C/75%RH

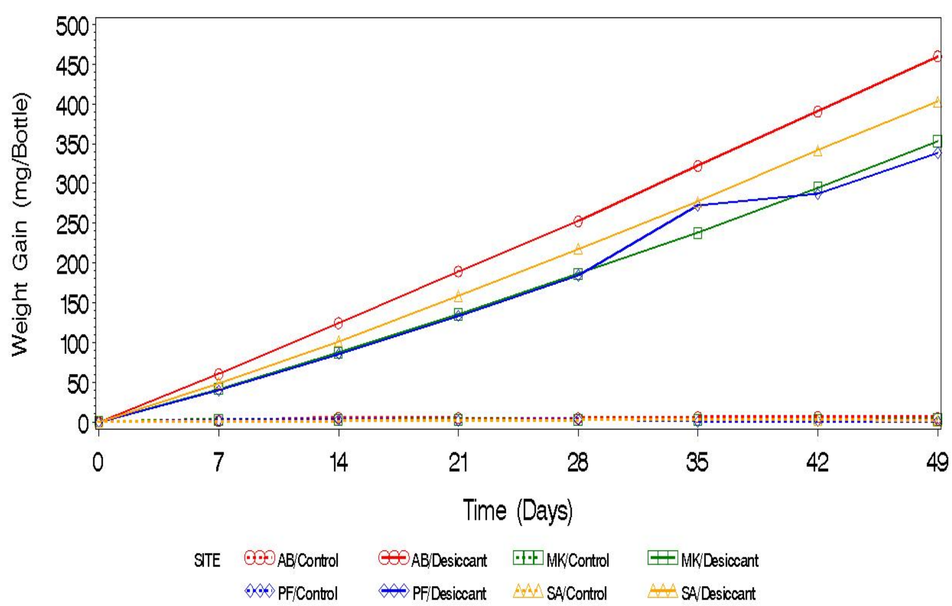
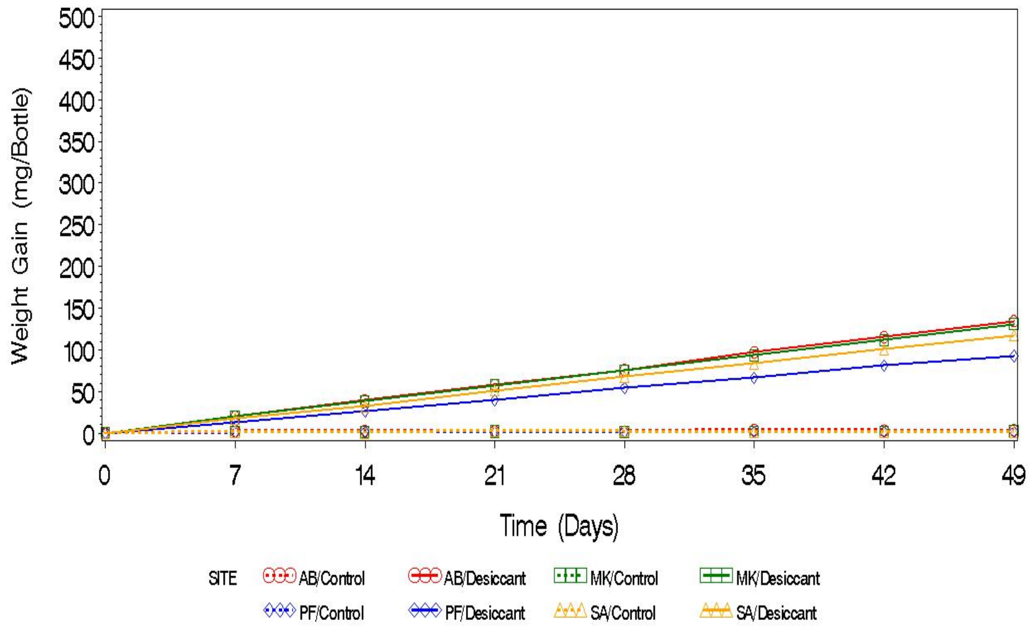


Figure 2
25°C/75%RH



Figures 3 and 4 show the mean weight gain (mg/bottle) profile (average of 15 bottles with desiccant or 10 control bottles) for intact bottles over the testing period for each lab. As can be seen in the profile plots, there is no apparent induction period and the weight gain profiles are linear over the testing interval. The intact bottles containing desiccant show an increase in weight over time at the higher temperature, but very little increase at the lower temperature. The controls show very little change over time.

Intact with Desiccant

Figure 3
40°C/75%RH

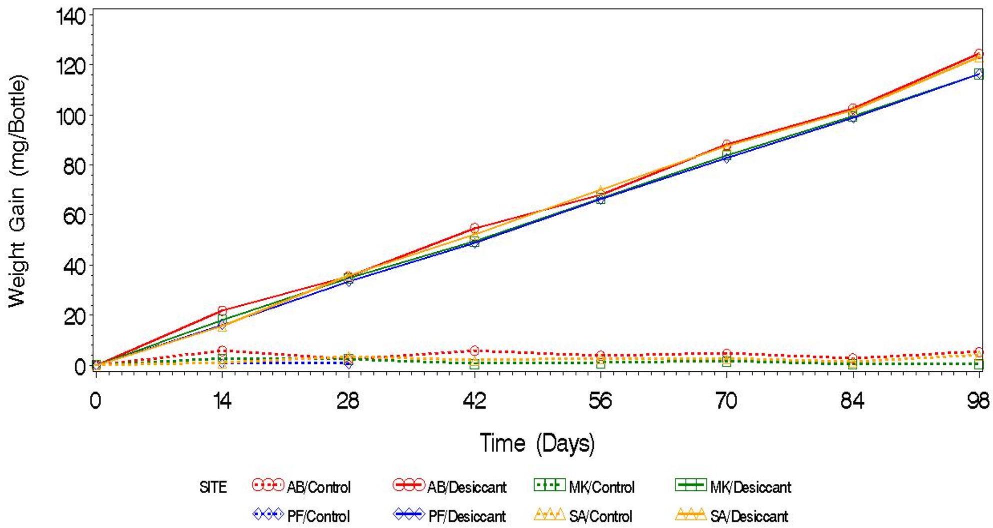
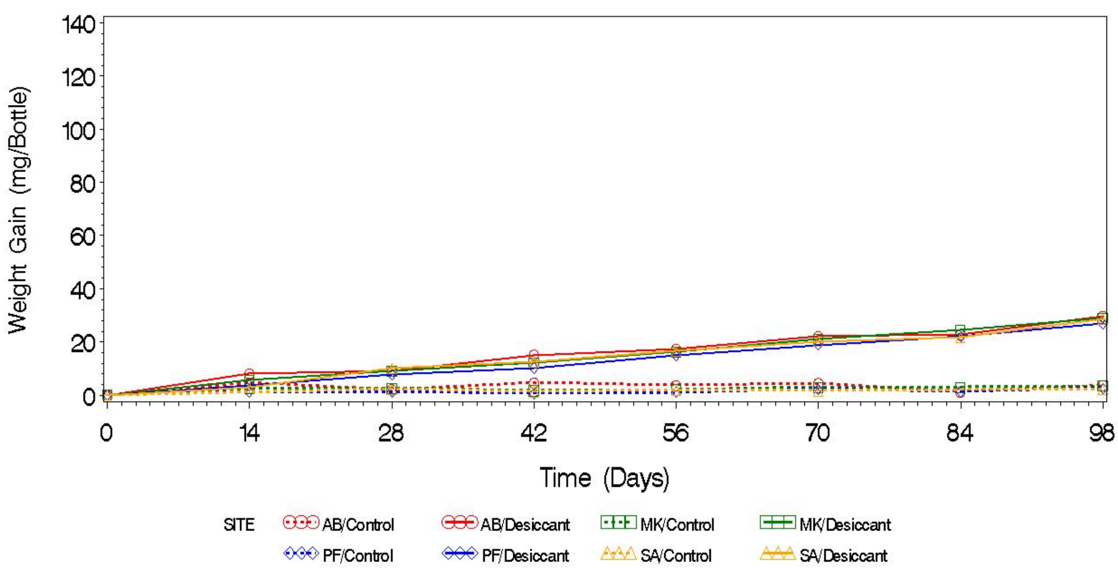


Figure 4
25°C/75%RH



Slopes compared for value and variability
A linear regression was fitted to the results for each container. The slopes (WVTR's) are plotted in Figures 5 and 6 for broached and intact bottles, respectively. The four labs show similar patterns within each temperature. . The containers having intact seals have smaller slopes and less variation than those having broached seals. Slopes for broached

and intact seals are plotted to the same scale in Figure 7, emphasizing the difference in variability between broached and intact seals.

Figure 5
Slopes (mg/day)
Broached

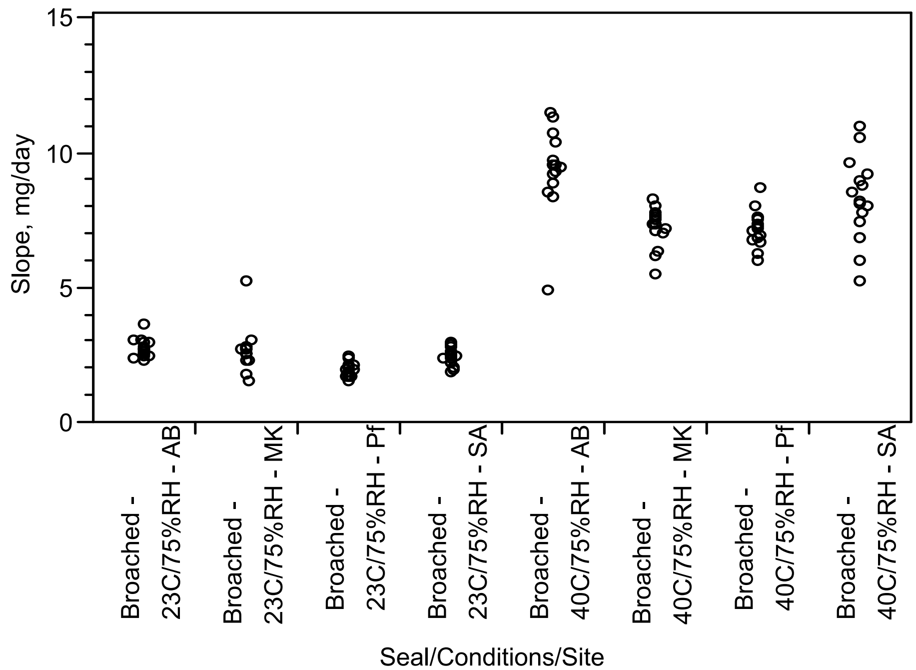


Figure 6
Slopes (mg/day)
Intact

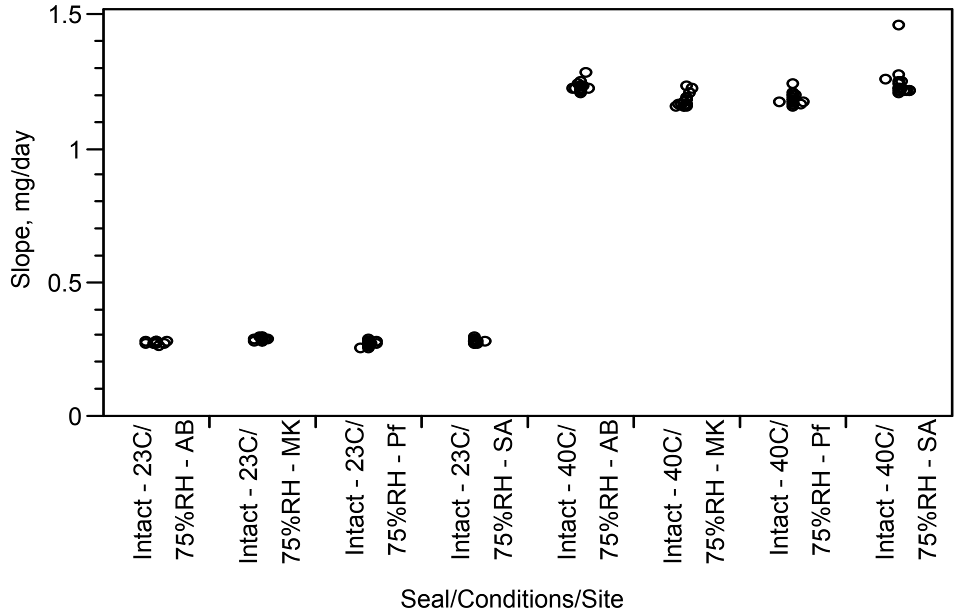
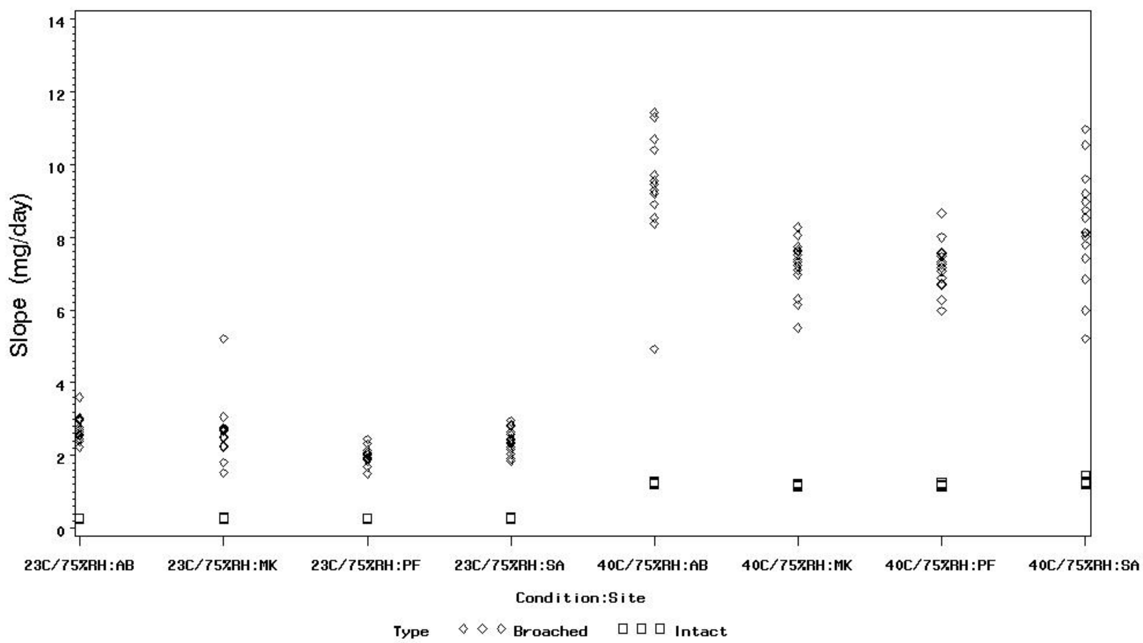


Figure 7
Slopes (mg/day)
Broached and Intact



Slope Summary
Tables 1 and 2 summarize the slopes for the broached and intact bottles. Included in these tables is the number of containers (N) in the analysis and the minimum and maximum slope across the N containers. The mean, standard deviation, and RSD (relative standard deviation) can be used to evaluate differences between sites as well as variability of the slopes. The p-value indicates whether or not the slope is significantly different from zero, i.e. a statistically significant result ($p < 0.05$) indicates that the slope was significantly different from zero. All slopes were significantly different from zero except one (controls at Pf at the higher temperature condition).

For the broached bottles (Table 1), note that the mean rate at 40/75 is similar across all laboratories (about 7 to 9.5 mg/day). The variability of the rates is much less at the lower temperature with respect to standard deviation but about the same with respect to RSD. See discussion of outliers below. Adjusting for the controls has little effect on the rate since the control slopes were so close to zero.

Table 1
Slope Summary
Broached

	Condition	Site	N	Mean Slope (mg/day)	Std Dev (mg/day)	RSD(%)	P-value	Min Slope (mg/day)	Max Slope (mg/day)
With Desiccant	40C/75%RH	MK	15	7.22	0.733	10.162	< 0.0001	5.523	8.290
		Pf	15	7.17	0.668	9.314	< 0.0001	5.982	8.679
		SA	15	8.28	1.549	18.701	< 0.0001	5.215	10.985
		AB	15	9.40	1.530	16.270	< 0.0001	4.934	11.445
	23C/75%RH	MK	15	2.65	0.809	30.496	< 0.0001	1.519	5.219
		Pf	15	1.92	0.262	13.664	< 0.0001	1.508	2.446
		SA	15	2.40	0.326	13.571	< 0.0001	1.853	2.949
		AB	15	2.74	0.338	12.332	< 0.0001	2.248	3.604
Control	40C/75%RH	MK	10	0.04	0.007		< 0.0001	0.028	0.047
		Pf	10	0.00	0.011		0.93	-0.019	0.016
		SA	10	0.05	0.007		< 0.0001	0.038	0.060
		AB	10	0.11	0.002		< 0.0001	0.109	0.116
	23C/75%RH	MK	10	0.03	0.009		< 0.0001	0.007	0.036
		Pf	10	0.03	0.005		< 0.0001	0.019	0.036
		SA	10	0.02	0.002		< 0.0001	0.019	0.025
		AB	10	0.06	0.003		< 0.0001	0.056	0.066

For the intact bottles (Table 2), the mean rate is similar across all laboratories within each temperature. The variability of the rates is much less at the lower temperature with respect to standard deviation but more similar with respect to RSD. Since the slopes are much smaller for the intact bottles with desiccant than the broached bottles, adjusting for the controls has more of an effect.

Table 2
Slope Summary
Intact

	Condition	Site	N	Mean Slope (mg/day)	Std Dev (mg/day)	RSD(%)	Test Slope = 0 P-value	Min Slope (mg/day)	Max Slope (mg/day)
With Desiccant	40C/75%RH	MK	15	1.18	0.026	2.170	< 0.0001	1.154	1.235
		Pf	15	1.19	0.022	1.820	< 0.0001	1.158	1.245
		SA	15	1.25	0.062	5.002	< 0.0001	1.210	1.460
		AB	15	1.23	0.018	1.470	< 0.0001	1.210	1.285
	23C/75%RH	MK	15	0.29	0.006	2.122	< 0.0001	0.276	0.297
		Pf	15	0.27	0.007	2.752	< 0.0001	0.257	0.283
		SA	15	0.28	0.006	2.122	< 0.0001	0.273	0.298
		AB	15	0.27	0.005	1.660	< 0.0001	0.264	0.281
Control	40C/75%RH	MK	10	-0.01	0.002		< 0.0001	-0.012	-0.007
		Pf	10	-0.02	0.005		< 0.0001	-0.031	-0.016
		SA	10	0.02	0.004		< 0.0001	0.018	0.028
		AB	10	0.02	0.005		< 0.0001	0.010	0.030
	23C/75%RH	MK	10	0.02	0.001		< 0.0001	0.023	0.025
		Pf	10	0.02	0.002		< 0.0001	0.015	0.023
		SA	10	0.01	0.004		< 0.0001	0.006	0.021
		AB	10	0.01	0.002		< 0.0001	0.010	0.016

Outliers

In three cases, the data appeared to be influenced by outliers. A discussion of those three situations follows:

From Table 1 and Figure 5:
Broached MK at 23/75, 30% RSD result: Container 4 had a slope of 5.22 mg/day. The other 14 slopes ranged from 1.52 to 3.07 mg/day. The Dixon outlier test indicates that the 5.22 is an extreme value (p-value < 0.001) assuming that the slopes are normally distributed. By leaving this point out, the MK mean, std dev, and RSD are 2.47, 0.40, and 16.3, respectively. The RSD calculated by omitting the outlier is in good agreement with the other site RSD's.

From Table 1 and Figure 5:
Broached AB at 40/75, 16% RSD result: Container 2 had a slope of 4.93 mg/day. The other 14 slopes ranged from 8.38 to 11.44 mg/day. The Dixon outlier test indicates that the 4.93 is an extreme value (p-value < 0.005) assuming that the slopes are normally distributed. By leaving this point out, the AB mean, std dev, and RSD are 9.72, 0.94, and 9.62, respectively.

From Table 2 and Figure 6:
Intact SA at 40/75, 5% RSD result: Container 6 had a slope of 1.46 mg/day. The other 14 slopes ranged from 1.21 to 1.28 mg/day. The Dixon outlier test indicates that the 1.46

is an extreme value (p-value < 0.001) assuming that the slopes are normally distributed. By leaving this point out, the SA mean, std dev, and RSD are 1.23, 0.02, and 1.72, respectively. The RSD calculated by omitting the outlier is in good agreement with the other site RSD's.

Nine Day Study
Since there was no apparent delay to reach steady state, Pfizer performed a 9 day study in both broached and intact bottles as well as controls. They used 15 test bottles and 10 controls, treated in the same way as the main study. The mean weights are given in figures 8-9 & 10-11 for broached and intact containers, respectively. For broached seals stored at 40°C/75%RH, there is no apparent induction period in the 9 day study. There is a short period of high slope during the first time period (day 0 to day 1) for the broached seals stored at 23°C/75%RH. For the intact seals, there is an extensive period of irregular behavior for the first six days of the 9 day study. Figures 10 and 11 show discontinuities and wide slope variation in the data for the whole six day period. For this nine day period, the performance is widely different among the four tests and irregular within two of them. This appears to be an induction period that was not evident in the main study.

Figure 8
Broached
40±2°C/75±5%RH

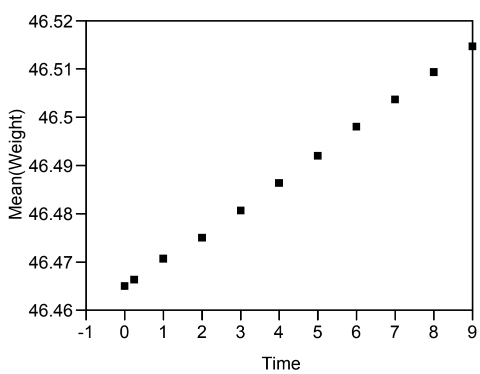


Figure 9
Broached
23±2°C/75±3%RH

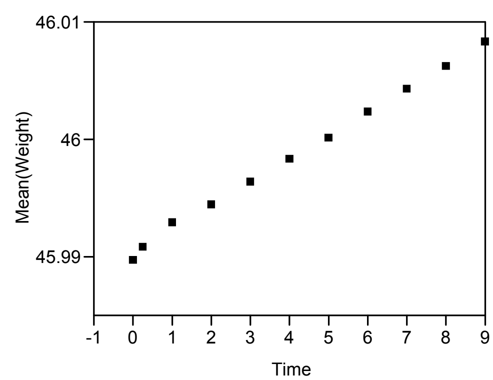


Figure 10
Intact
40±2°C/75±5%RH

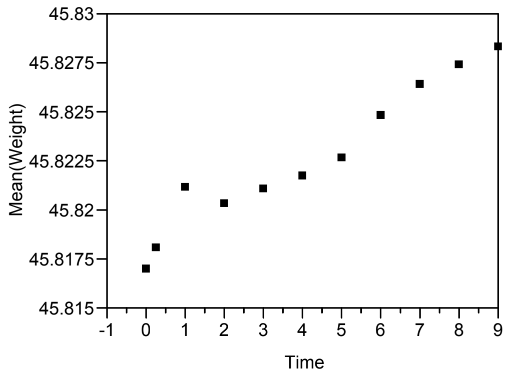
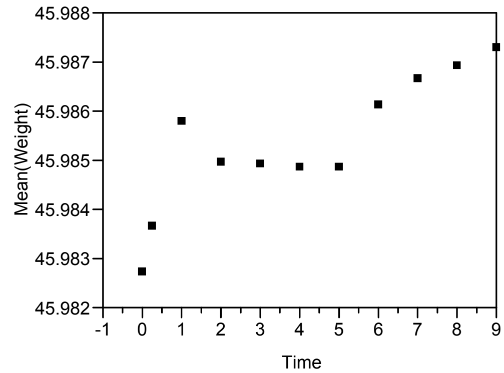


Figure 11
Intact
23±2°C/75±3%RH



Impact of Controls on Slopes

Tables 3 and 4 compare the mean slopes (WVTRs) for broached and intact containers at the two test conditions. In both tables, the overall slopes adjusted for controls at both conditions are 94 % or better of the slopes for desiccant filled bottles WVTRs obtained for desiccant filled bottles alone are slightly higher than WVTRs obtained when controls are subtracted as specified in USP <671>.

Table 3
Mean Slopes (mg/day) for Broached Seal

	40C/75%RH			23C/75%RH		
Site	Desiccant	Adjusted for Control	Ratio (%)	Desiccant	Adjusted for Control	Ratio (%)
MK	7.216	7.176	99.4	2.653	2.626	99.0
Pf	7.173	7.173	100.0	1.917	1.892	98.7
SA	8.283	8.233	99.4	2.399	2.377	99.1
AB	9.403	9.291	98.8	2.739	2.679	97.8
		Overall	99.4		Overall	98.6

* Ratio = Adjusted for Control slope as a percent of Desiccant slope

Table 4
Mean Slopes (mg/day) for Intact Seal

Intact	40C/75%RH			23C/75%RH		
Site	Desiccant	Adjusted for Control	Ratio*	Desiccant	Adjusted for Control	Ratio*
MK	1.179	1.188	100.8	0.286	0.262	91.6
Pf	1.186	1.208	101.9	0.271	0.252	93.0
SA	1.246	1.223	98.2	0.280	0.269	96.1
AB	1.230	1.208	98.2	0.275	0.263	95.6
		Overall	99.7		Overall	94.1

* Ratio = Adjusted for Control slope as a percent of Desiccant slope

Comparison of RSDs; Effect of Temperature on Variation
Table 5 compares the RSD's for broached and intact containers with desiccant at the two storage conditions. Considering the effect of outliers discussed above, the variation for broached containers at 40°C/75%RH (mean =15.9) is slightly higher than at 23°C/75%RH (mean=13.9). Otherwise, the variations are similar, and there is little difference in RSDs between the two conditions.

Table 5
RSD (%)*

	40C/75%RH		23C/75%RH	
Site	Broached	Intact	Broached	Intact
MK	10.16	2.17	30.50 (16.33)	2.12
Pf	9.31	1.82	13.66	2.75
SA	18.70	5.00 (1.72)	13.57	2.12
AB	16.27 (9.62)	1.47	12.33	1.66

* RSD without outlier in parentheses

Variation Between Sites, Runs and Containers
Table 6 is an analysis of the RSDs. It breaks the overall RSD into two components; the variation associated with 'Between Sites and Runs' and that associated with 'Between Containers'. The least variation occurs when intact and broached containers are tested at 40°C/75%RH, using regression analysis over all five data points. Using initial point to 14 days shows similar mean WVTRs, but in most analyses the variation is larger for the initial to day 14 calculation than using regression. The RSDs for WVTR calculated using the data from initial to day 14 at 23°C/75%RH are very large .

Table 6
Comparison of Type, Condition, and Slope Method Using RSD

Type	Condition	Slope Method	Mean Slope (mg/day)	RSD(%) Between Sites&Runs	RSD(%) Between Containers	RSD(%) Total
Broached	23C/75%RH	Initial - Day 14	2.44	16.44	23.92	29.02
		Regression 1 (all)	2.43	14.31	19.99	24.58
		Regression 2 (wo Initial)	2.42	14.06	19.40	23.96
		Regression 3	2.42	13.50	19.93	24.07
	40C/75%RH	Initial - Day 14	7.12	17.56	17.43	24.74
		Regression 1 (all)	8.02	12.59	14.92	19.52
		Regression 2 (wo Initial)	8.18	11.96	14.52	18.82
		Regression 3	8.14	10.89	14.99	18.53
Intact	23C/75%RH	Initial - Day 14	0.36	44.36	5.97	44.76
		Regression 1 (all)	0.28	2.35	2.18	3.20
		Regression 2 (wo Initial)	0.27	4.15	2.34	4.77
	40C/75%RH	Initial - Day 14	1.28	15.59	3.47	15.97
		Regression 1 (all)	1.21	2.63	3.02	4.00
		Regression 2 (wo Initial)	1.21	2.72	3.39	4.34

Notes: (1) Initial-Day 14 is day point 0 and day point 14. (2) Regression 1 is regression over days 0 to 42 or 49. (3) Regression 2 is regression over days 7 to 42 or 49. (3) Regression 3 is regression on days 7, 14, 21, 28, and 35; not much different from using 42 or 49 days.

Conclusions

- There was no apparent induction period in the main study; steady state appears to have occurred by the 7 day time point.
- The 9 day study showed irregularity and variable behavior during the first 6 days, suggesting instability during this period which did not appear in the test as specified by USP Chapter <671>.
- Weight gain was linear over time at steady state; WVTR is constant.
- Smaller variability occurred when WVTR was calculated by regression rather than using the two- point calculation of USP <671>.
- Mean WVTR rates and container variability were similar between labs within each of the testing conditions.
- The higher temperature resulted in higher WVTR rates. RSD's were similar between the two temperatures.
- Controls (blanks) had statistically significant but small positive slopes. In this study, the controls had little effect on the WVTR rates for the broached bottles.

Controls had a greater effect on the intact bottle because of the smaller intact WVTR rates.

- Controls are not necessary (see Table 3).
- Anhydrous calcium chloride should be pre-dried at 210° C, not 110° C as is currently specified in USP <671> (see Appendix).

Recommendations

- USP Chapter <671> should be rewritten or replaced.
- For the purpose of moisture-barrier comparison, WVTR testing should be conducted at 40°C/75% RH. This is an ICH accelerated condition.
- The test duration should be 35 days, and containers weighed at 7 day intervals
- WVTR should be calculated by regression analysis over the period from day 7 to day 35.
- Blanks (Controls) are not necessary, so they should not be included in the test design
- If calcium chloride is used as the desiccant, it should be pre-dried at 210°C

Appendix A

Selection of desiccant for bottle WVTR study

Anhydrous calcium chloride is used as a desiccant for measuring the WVTR of HDPE bottles. This desiccant is specified in USP<671> with a requirement that the desiccant be activated at 110°C prior to use. The amount of the desiccant to use is also specified in USP<671>, depending on container sizes. The purpose of using desiccant is to provide a sink condition; hence a constant driving force of water vapor pressure over the course of study at constant temperature and external humidity. For the current study, a sink condition is defined as an internal relative humidity that does not exceed 5% over the time course of study. To assure this criterion is met, the following procedures were carried out to determine the suitable amount of the desiccant to use:

1. A literature search was conducted for information on the properties of anhydrous chloride, including moisture sorption isotherms
2. The cumulative amount of water vapor that will permeate into the container during the study was estimated.
3. The desiccant isotherm was linked with the estimated bottle WVTR to verify that the criterion of not more than 5% RH is met.

Data in the literature shows that the relative humidity in air dried by anhydrous calcium chloride is 1.6% at 25°C[i]. Literature also indicates that calcium chloride can exist in multiple hydrates, and that the hexahydrate loses water at 200°C[ii]. This information on hydrates shows that the activation temperature must be higher than 200°C if the desiccant has been previous exposed to humidity and formed hexahydrate. The history of the desiccant must be considered because during shipping, storage, and use the anhydrous calcium chloride may be exposed to moisture at some points, hence care must be taken to activate the material properly before use.

A preliminary experiment was conducted by a member laboratory to select the activation temperature for anhydrous calcium chloride after the material was equilibrated at 6.4%RH at room temperature. Activation was conducted by heating at 110°C and 210°C. Results showed that loss on drying was 0.5% and 4.5% by activation at 110°C and 210°C, respectively. These results showed that activation of anhydrous calcium chloride at 210°C is necessary to fully activate the desiccant even if the material has been exposed to an environment of extremely low humidity.

To estimate the quantity of anhydrous calcium chloride needed to maintain a sink condition for study, the desiccant moisture capacity was linked to the estimated quantity of moisture ingress during the study. The moisture capacity of the desiccant activated at 210°C is about 2% at 5%RH. The WVTR of the intact 60 mL bottle was estimated to be 0.2mg/day at 23°C/75%RH, and 0.9mg/day at 40°C/75%RH. Total ingression of moisture over 98 days is 19.6mg and 88mg under the two study conditions. It can be calculated that about 5g of fully activated anhydrous calcium chloride is needed to absorb 88 mg of the moisture penetrating the container to result in an internal relative humidity of about 5%. In the current study, 30 g of fully activated anhydrous calcium chloride was used for the 60 mL bottle. This amount of desiccant ensured that a sink condition of <5%RH was maintained throughout the study. This quantity also provides a sink condition for the broached bottle over a 49 day study (Table A1).

Table A1. Estimation of relative humidity in the headspace of 60 mL HDPE bottles

40C/75%RH	Actual WVTR mg/day	Test Duration Days	Total Water Ingressed, mg	Amount of Desiccant, g	Water/Desiccant, % (w/w)	Headspace RH [iii] %
Intact	1.25	98	122.5	30	0.4%	<5%
Broached	9.4	49	460.6	30	1.5%	<5%
23C/75%RH						
Intact	0.29	98	28.42	30	0.1%	<5%
Broached	2.74	49	134.26	30	0.4%	<5%

i. G.P. Baxter, H.W. Starkweather, 1916. The efficacy of calcium chloride, sodium hydroxide and potassium hydroxide as drying agents. J. Am. Chem. Soc., 38, 2038-2041.

ii. S. Budavari, M.J. O'Neil, A. Smith, P.E. Hekelman. The Merck Index, 11th Ed. Merck & Co., Inc., Rahway, N.J., 1989. p 252.

iii. Y. Chen, Y. Li, Determination of water vapor transmission rate (WVTR) of HDPE bottles for pharmaceutical products. Int. J. Pharm., 358 (2008) 137-143.

Appendix B

Sample Preparation and Handling

Excerpted from PQRI Bottle WVTR Study Protocol, 5 September, 2006

Sample Preparation at the Manufacturing Plant

Desiccant

- Desiccant should be taken from the original container and opened just prior to use. Place bulk desiccant in shallow container, taking care to exclude any fine powder. Dry at 210 degree Celsius for a minimum of one hour and cool in a glass desiccator for at least 30 minutes. The desiccator should be kept closed during packaging of the bottles. No desiccant should be exposed to the ambient environment unprotected for more than 10 min

Bottles with Desiccant

- To each of >240 bottles, add 30+-3.0 g of the desiccant. Immediately close the bottles with the S&T CR cap, manually without significant torque.
- Using a calibrated instrument, close the bottles with the cap in accordance with the procedure described in USP <671>. Activate the seal with the induction sealer. The induction seal must pass ASTM D3078-02 at 15 inches mercury (or equivalent) vacuum-dye leak test.
- Mark each bottle sequentially 1-240

Empty Bottles

- Follow the same procedure as described above and prepare >160 induction-sealed bottles without any desiccant.
- Instead of desiccant add glass glass beads per USP<671> to the same volume as the desiccant
- Mark each bottle sequentially 1-160

Shipping of Sample to Testing Labs

- Place at least 60 (e.g. 70) bottles containing desiccant and at least 40 (e.g. 50) empty bottles (with caps) in an appropriate container and FedEx them to the 4 testing labs within 3 days.

Sample Preparation and Weight Measurement at the Testing Labs

Intact Bottles

- Separate 60 bottles-with-desiccant into two equal parts, A & B mark them with non-erasable ink (e.g. A1, B2, etc.)
- Measure and record the initial weight to the 4th decimal point (0.1 mg) of each bottle in group A (30 in total). Place 15 group-A bottles each in the 40°C and 23°C chambers, respectively. Measure and record the weight of each bottle every 14 days for 98 days, to the 4th (0.1 mg). Before each weighing, the bottles are removed from the chamber and allowed to equilibrate for ~30 min in the room (preferably with T/RH control to 75F (24°C)/40%RH or below) where the weighing takes place. Minimize the time-out-of-chamber to below 2 hrs.
- Mark the 40 empty bottles with non-erasable ink as C1, C2, etc.
- Place 10 empty bottles with intact seal in each of the environmental chambers at the same time when the desiccated bottles are placed (20 bottles in total).
- Measure and record the weight of each of the empty bottles as for the desiccated bottles.

Breached Bottles

- Breach the seal of group-B bottles (30 in total) according to the attached procedure. Close these bottles with the cap according to the prescribed procedure (USP <671>).
- Weigh and record the weight of each of the group-B bottles with breached seal to the 4th decimal point (0.1 mg). Store 15 bottles each in the 23° and 40° chambers, respectively, as with the intact bottles. Measure and record the weight of each bottle to the 4th decimal point (0.1 mg) every 7 days for 49 days. Before each weighing, the bottles are removed from the chamber and allowed to equilibrate for ~30 min in a room (preferably with T/RH control to 75F (24°C)/40%RH or below) where the weighing takes place.
- Breach the seal of the remaining 20 empty bottles according to the prescribed procedure. Close these bottles with the cap according to the prescribed procedure (USP <671>).
- Place 10 empty bottles with breached seal in each of the environmental chambers at the same time when the desiccated bottles are placed.
- Measure and record the weight of each of the empty bottles as for the desiccated bottles.

Appendix B
Procedure for breaching the induction-seal and preparing the breached bottle samples

Excerpted from PQRI Bottle WVTR Study Protocol, 5 September, 2006

The induction inner-seal must be breached by testing laboratory in a consistent manner. The testing laboratory must puncture the seal using either a razor blade or a conical instrument, leaving a flat consistent inner-seal residue on the lip of the bottle. After breaching, the bottles must be closed and opened with caps for 30 times and re-torque at the end in accordance with the procedure described in USP <671>, MULTIPLE-UNIT CONTAINERS FOR CAPSULES AND TABLETS.