

CASE STUDIES

Methods and Considerations for Disposable Implementation

Design, Compliance, and Validation

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A CASE STUDY OF EXTRACTABLE TESTING

An extractables testing program was initiated to estimate the quantity of chemicals that can potentially leach from plastics into process streams. Model test solutions were used to bracket the process solutions.

The purpose of this study was to

- Quantify the amount of extractables generated by storing process-specific solutions in biobags from Vendors A and B and in PETG bottles (glycol-modified polyethylene terephthalate, a copolyester)

- Generate a worst-case estimate for the maximum amount of extractables that could be concentrated with the product and evaluate the risk associated with the worst-case extractability findings.

- Ensure that process solutions were chemically compatible with the containers they are stored in.

- Recommend storage criteria for each process solution evaluated, based on the extraction results of each model process solution.

An extractable study is conducted by filling test bags or bottles with the process solutions to be evaluated. A 500-mL to 1-L test container volume is typically chosen to maximize the surface-to-volume ratio of the test container-solution combination. The test containers are then stored for the actual storage time that will be used in production. Entire test containers are harvested and their contents

Equation 1

$$\frac{\text{Total mg extractables per batch}}{\text{Minimum doses per batch}} = \frac{\text{Total mg extractables}}{\text{per dose}}$$

Equation 2

$$C_{\text{Larger}} = C_{\text{Test}} \left(\frac{SV_{\text{Larger}}}{SV_{\text{Test}}} \right)$$

Equation 3

$$\frac{\text{Amount of acetonitrile}}{\text{Batch}} = \left(100 \frac{\text{mg of acetonitrile}}{\text{L}} \right) \left(200 \frac{\text{L solution}}{\text{Batch}} \right) = 20,000 \frac{\text{mg acetonitrile}}{\text{Batch}}$$

$$\frac{\text{Amount of acetonitrile}}{\text{Amount of drug}} = \left(\frac{20,000 \frac{\text{mg of acetonitrile}}{\text{Batch}}}{1,000,000 \frac{\text{mg of drug}}{\text{Batch}}} \right) = 0.02 \frac{\text{mg Acetonitrile}}{\text{mg Drug}}$$

Equation 4

$$\frac{\text{Amount of acetonitrile}}{\text{Day}} = \left(0.02 \frac{\text{mg of acetonitrile}}{\text{mg of drug}} \right) \left(100 \frac{\text{mg Drug}}{\text{Day}} \right) = 2.0 \frac{\text{mg of acetonitrile}}{\text{Day}}$$

Equation 5

$$Q_{10}^{((40^{\circ}\text{C}-25^{\circ}\text{C})/10)} = 1.8^{1.5} = 2.415 = \text{AAF}$$

analyzed at intermittent times during the study.

For this study, we used a worst-case approach to estimate the maximum amount of extractables that could be carried into a product stream. We then evaluated the risk associated with those findings. Our assumptions for the worst-case model were that

- All extractables leaching into the process stream would be carried through to the final product; no

extractables would be removed using the process purification steps.

- If samples were taken at multiple time points, the time point with the highest extractable concentration was used for estimates.

- The solution with the highest total extractable concentration observed during the testing would be used to estimate the worst-case extractables dosage; for instance, if WFI extracted more than acids and bases, then the results for WFI were

used as the results for all solutions.

- The biobag with the highest extractables (Vendor A or B) was used for the worst-case extractables dosage estimate, thereby qualifying both bag vendors.

We compared our worst-case dosage estimate for extractables with the ICH guidelines for extracted materials. ICH has published a set of guidelines recommending the maximum allowable dosages and concentrations of extractables based on the toxicological data of chemical species. These guidelines were established by evaluating solvents that typically are used in the manufacture of plastics. The guidelines categorize extractables into three classes: Class I, Class II, and Class III (5, 7).

Class I extractables are those chemicals that should be avoided. They pose serious health risks at even the lowest concentrations (benzene, for example).

Class II extractables are suspected of some levels of toxicity and should be limited. Each Class II extractable has its own toxicity specification, which can be from 0.5 mg/day to 50.0 mg/day, with a total solution concentration ranging from 50 to 5000 ppm.

Class III extractables have low toxic potential. The maximum dosage of a Class III extractable that an individual can receive under ICH guidelines is 50 mg/day with a total solution concentration of no more than 5000 ppm.

A toxicologist should evaluate any additional compounds that are identified in your study to determine the potential risk they pose.

DATA ANALYSIS

Test results for extracted compounds were totaled and compared to ICH guidelines for Class I, II, and III residual solvents on a per dose of drug product basis according to the following calculation in Equation 1.

Using this worst-case model, the extractable estimate was generated by the following sequence of steps:

Step 1: Using the raw data tables, we calculated the highest concentration of total extractables for each solution and bioprocess container.

Table 1: Biobags and bottles used for storage of solutions at an Amgen facility

Container Type	Primary Vendor/Part #	Secondary Vendor/Part #	Solution Stored	Storage Conditions/ Classification
5-L pillow bag	Vendor A AMG5L8XM	Vendor B AMG- 8-5LF-A	Inoculation media	2–8 °C, Class 100,000
	Vendor A AMG5L6XNAC	Vendor B AMG- 6-5LFF-A	Sodium carbonate	Ambient, Class 100,000
10-L pillow bag	Vendor A AMG10L8XM	Vendor B AMG- 8-10LF-A	Inoculation media	2–8 °C, Class 100,000
			Sodium carbonate	Ambient, Class 100,000
50-L bag	Vendor A AMG50L3D	Vendor B AMG- 50FF-A	Inoculation media	2–8 °C, Class 100,000
100-L bag	Vendor A AMG100LF22	Vendor B AMG- 100FSB-A	500mM Tris	Ambient, Class 100,000
	Vendor A AMG100LF1	Vendor B AMG- 100F-A	Amino acid C	Ambient, Class 100,000
			Glucose	Ambient, Class 100,000
200-L bag	Vendor A AMG200L3D	Vendor B AMG- 200F-A	Inoculation media	2–8 °C, Class 100,000
			Amino acid B	Ambient, Class 100,000
	Vendor A AMG200LSR	Vendor B AMG- 200FB-3D-A	Sodium hydroxide	Ambient, Class 100,000
250-L bag	Vendor B AMG-250TC- 3D-A	NA	Calf serum	2–8 °C, Class 100,000
500-L bag	Vendor A AM500NAC	Vendor B AMB- 500FB-3D-A	Sodium carbonate	Ambient, Class 100,000
	Vendor A AM500RIM	Vendor B AMG- 500FB34-3D-A	Regeneration buffer	Ambient, Class 100,000
			Column storage buffer	Ambient, Class 100,000
750-L bag	Vendor B AMG-750TC- 3D-A	Vendor A AM750LCHR2	Calf serum	2–8 °C, Class 100,000
	Vendor B AMG-750MF- 3D	Vendor A AM750LCHR	Inoculation media	2–8 °C Class 100,000
500-mL PETG bottle	Vendor C 322020-0500	NA	Inoculation media	2–8 °C, Class 100,000
			IGF-1	2–8 °C, Class 100,000
2-L PETG bottle	Vendor C 322020-2000	NA	Hydrocortisone solution	</= –10 °C

This was done for both Class II and Class III ICH compounds. No Class I ICH compounds were identified during the study.

Step 2: We calculated the volume of process solution that was expected to be stored in each bioprocess container per bulk drug substance (BDS) batch.

Step 3: We adjusted the extractable concentrations from Step 1 using the surface-area-to-volume ratios of the extractable study test containers and the process containers. (Note: The

details of this concentration adjustment method are described in Standard ISO 10993-12, “Sample Preparation and Reference Materials”) (8). This standard addresses the issue of proving the safety of medical devices by identifying various types of biocompatibility tests.)

To calculate extractable concentrations for a larger container, the surface to volume ratios were scaled from the extractable study test container to the larger container as

Table 2: Validation results

Container	Vendor	Run #	Study Start (day 0 sample)	Sample Date	Acceptance Criteria	Results (cfu/10 mL)
10-L pillow bags	Vendor B	1	07-12-04	07-12-04	≤50 cfu/10 mL	0
				07-28-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	0
		2	07-12-04	07-12-04	≤50 cfu/10 mL	0
				07-28-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	0
	3	07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-28-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
	Vendor A	1	07-12-04	07-12-04	≤50 cfu/10 mL	0
				07-28-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	0
2		07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-28-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
09-28-04*	≤50 cfu/10 mL	NA				
09-30-04*	≤50 cfu/10 mL	0				
3	07-12-04	07-12-04	≤50 cfu/10 mL	0		
		07-28-04	≤50 cfu/10 mL	0		
		08-19-04	≤50 cfu/10 mL	0		
750-L regular bags	Vendor B	1	07-14-04	07-14-04	≤50 cfu/10 mL	0
				07-29-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	0
		2	07-14-04	07-14-04	≤50 cfu/10 mL	0
				07-29-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	10
	09-28-04	≤50 cfu/10 mL	0			
	3	07-14-04	07-14-04	≤50 cfu/10 mL	2	
			07-29-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
			09-28-04	≤50 cfu/10 mL	0	
			07-29-04	≤50 cfu/10 mL	0	
08-19-04			≤50 cfu/10 mL	0		
09-28-04	≤50 cfu/10 mL	0				
750-L regular bags	Vendor A	1	07-12-04	07-12-04	≤50 cfu/10 mL	0
				07-29-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	0
	2	07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-29-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
	3	07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-29-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
09-28-04	≤50 cfu/10 mL	0				
2-L PETG bottles	Vendor C	1	07-12-04	07-12-04	≤50 cfu/10 mL	0
				07-28-04	≤50 cfu/10 mL	0
				08-19-04	≤50 cfu/10 mL	10
	2	07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-28-04	≤50 cfu/10 mL	0	
			08-19-04	≤50 cfu/10 mL	0	
	09-28-04	≤50 cfu/10 mL	0			
	3	07-12-04	07-12-04	≤50 cfu/10 mL	0	
			07-28-04	≤50 cfu/10 mL	0	
08-19-04			≤50 cfu/10 mL	0		
09-28-04	≤50 cfu/10 mL	0				

shown in Equation 2, where
 C_{test} = Concentration of extractable study test sample

C_{larger} = Concentration of larger process container sample

SV_{Test} = Surface-to-volume-ratio of extractable study test container

SV_{Larger} = Surface-to-volume-ratio of larger process container

Step 4: We then multiplied the adjusted maximum extractable concentrations (Step 3) by the corresponding volume for each bag/bottle (Step 2) and summed them to get the total mass of extractables per BDS batch.

Step 5: The total amount of extractables per BDS batch (Step 4) was divided by the average batch size to get the weight of extractables per weight of drug molecule.

Step 6: The weight fraction of extractables to drug (Step 5) was multiplied by “X” mg drug per day to arrive at the maximum amount of extractables per day that a dose could possibly contain under the worst-case model.

Step 7: The dosage figure obtained in (Step 6) was divided by the ICH guideline (0.5 mg/day for Class II and 50 mg/day for Class III), and the result was multiplied by 100% to compare to the guideline.

HYPOTHETICAL EXAMPLE

Consider the following hypothetical example using the first two listed Class II and Class III ICH chemicals in the guideline (the numbers used here have been fabricated to demonstrate only the approach used to generate an extractable estimate).

A drug product is manufactured that produces 1 Kg of product per batch. A patient receives 100 mg/day of the drug as a typical dose. One feed solution to the process uses two volumes of 100 L each for a total of 200 L. The manufacturer wants to use two polyethylene biobags with capacities of 100 L and to store the solution for a maximum of four months before use. A four-month extractable study is conducted, and the results are shown in Table 3.

Two extractables were identified: acetonitrile, a Class II extractable at a maximum concentration of 100 mg/L;

and acetone, a Class III extractable at a maximum concentration of 500 mg/L. The hypothetical concentrations of 100 ppm and 500 ppm are below the ICH concentration limits, meeting one of the test criteria. The extractable evaluation for the ICH-permitted daily exposure (PDE) limit would then proceed as shown in Equation 3.

If a hypothetical dose of the drug is 100 mg/day, the maximum dose of acetonitrile that could be delivered with the drug is as shown in Equation 4.

The daily exposure limit for acetonitrile is 4.1 mg/day, so this estimate would be within the ICH guideline for this Class II solvent. The calculation can be repeated for the case of the Class III compound acetone and would result in a dose of 10.0 mg/day, which is within the ICH guideline PDE limit of 50 mg/day for this Class III solvent.

It is important to note that extractables studies such as this generate worst-case estimates and do not consider the effect of downstream purification processes to clear any extractables.

EXECUTION OF EXPERIMENTS

Table 4 outlines a list of solutions and materials tested for extractables. Amgen, Inc., supplied a testing facility with the raw materials required to formulate the designated manufacturing solutions in Table 2. The testing facility supplied the WFI required to formulate the manufacturing solutions. Also, the testing facility prepared the test solutions using batch sheets supplied by Amgen, Inc.

The manufacturing solutions were stored in the following containers supplied by Amgen, Inc.:

- 500-mL Vendor A biobags
- 1000-mL Vendor B biobags
- 500-mL Vendor C PETG bottles
- 500-mL and 1-L polypropylene bottles.

The characterization study lasted for 72 days. Part of the study was performed with aqueous solutions under accelerated conditions at 40 °C. Accelerated sample time points were taken at T = 0, 14, and 30 days as listed in Table 1. The rest of the study

Table 3: Results of a hypothetical extractable study

Extractable	CAS Number	ICH Class	Hypothetical Concentration, mg/L (ppm)	ICH Concentration Limit, mg/L (ppm)	ICH PDE ¹ Limit, mg/day
Acetonitrile	75-05-8	2	100	410	4.1
Acetic Acid	64-19-7	3	500	5000	50

¹PDE = permitted daily exposure

Table 4: Solutions and materials tested for extractables

Test Article	Solution	Test Article Size (mL)	Fill Volume (mL)	Time Points (Day)	Aging Conditions
Vendor A bag	D	500	250	0, 14, 30	40 °C
Vendor B bag	A, B, C, D	1000	500	0, 14, 30	40 °C
PP bottles	A, B, C	500	500	0, 14, 30	40 °C
PP bottles	E	500	500	0, 34, 72	25 °C
PP bottles	F	1000	500	0, 14, 30	40 °C
PETG bottles	A, B, C	500	500	0, 14, 30	40 °C
PETG bottles	E	500	500	0, 34, 72	25 °C

Solution A = water for injection (WFI)
 Solution B = 2 M sodium hydroxide
 Solution C = 1 M hydrochloric acid
 Solution D = 1 M sodium carbonate
 Solution E = 95% ethanol/5% water
 Solution F = autoclaved water for injection (WFI)

Table 5: Matrix of analytical tests conducted during the production extractable study

Solution	Media/Buffer	pH	Conductivity	TOC	Metals	Volatiles	Semi-volatiles	Non-volatiles
A	Water for injection (WFI)	X	X	X	X	X	X	X
B	2 M sodium hydroxide	X	X		X	X	X	X
C	1 M hydrochloric acid	X	X		X	X	X	X
D	1 M sodium carbonate	X	X		X	X	X	X
E	95% ethanol/5% water		X		X	X	X	X
F	Autoclaved water for injection	X	X	X	X	X	X	X

was performed with ethanol solutions under normal aging conditions at 25 °C. Normal sample time points were taken at T = 0, 34, and 72 days.

Accelerated Aging: The aqueous samples and controls for days 14 and 30 were stored at 40 °C as an accelerated aging condition. Accelerated aging is performed by storing articles at elevated temperatures for extended periods of time and mathematically correlating increased temperature with time (known as the acceleration factor, Q_{10}). The FDA-recognized consensus standard value for Q_{10} is 1.8 for each

increase of 10 °C over ambient temperature (25 °C), to a maximum of 35 °C above ambient. An accelerated aging factor (AAF) is calculated as Q_{10} raised to the power of the storage temperature minus ambient temperature divided by 10, as seen in Equation 5.

Accelerated age time (AAT) for this study represents the maximum time that samples are maintained under the accelerated aging condition. The real-time equivalent age of samples and controls for each time point are calculated as

Table 6: Results of pH measurement during the extractable study

Test Material and Test Solution	Solution pH		
	Day 0	Day 14	Day 30
Vendor B, water for injection (WFI)	8.2	6.6	4.7
PETG bottle, WFI	8.9	6.2	7.4
Polypropylene bottle, WFI	8.6	6.3	7.3
Control, WFI	7.8	6.3	7.3
Vendor B, 2 M sodium hydroxide	14	14	14
PETG bottle, 2 M sodium hydroxide	14	14	14
Polypropylene bottle, 2 M sodium hydroxide	14	14	14
Control, 2 M sodium hydroxide	14	14	14
Vendor B, 1 M hydrochloric acid	<1	<1	<1
PETG bottle, 1 M hydrochloric acid	<1	<1	<1
Polypropylene bottle, 1M hydrochloric acid	<1	<1	<1
Control, HCL	<1	<1	<1
Vendor A, 1 M sodium carbonate	12	12	12
Vendor B, 1 M sodium carbonate	12	12	12
Control, 1 M sodium carbonate	12	12	12
Polypropylene bottle, autoclaved WFI	8.2	10	10
Control, autoclaved WFI	8.5	7	9

$$AAT \times AAF = \text{real-time equivalent age}$$

Therefore, after 14 days of storage at 40 °C, the day 14 samples and controls will have a calculated real-time equivalent age of 33.7 days at 25 °C (14 days × 2.41). After 30 days of storage at 40 °C, the Day 30 samples and controls will have a calculated real-time equivalent age of 72.4 days at 25 °C (30 days × 2.415).

Samples from each time point are to be analyzed according to the analytical matrix in Table 5.

RESULTS AND DISCUSSION

The results of the extractables study are detailed below. First, the results for each test method are presented and discussed. Then the results of the worst-case extractables dosage estimates are reviewed.

Solution pH: For most of the samples, there was no significant change in solution pH over the course of the study (Table 6). The variation observed in pH for WFI is not of concern, because the solution has no buffering capacity and will not serve as a buffer in the drug process

Solution Conductivity: The results

of conductivity measurements are listed in Table 7 for the aqueous solutions in the study. The target values observed were as expected, and no significant drifts in conductivity were recorded.

Metals Analysis: The metals content was measured to determine whether the test solution extracted metals from the contact layer of the test bag/bottle. Samples and controls using Solution B (sodium hydroxide) and Solution D (sodium carbonate) were not evaluated for sodium because of its high concentration in the solution. The results are shown in Table 8.

The trace levels of sodium observed in Table 8 are not of concern because sodium is an inherent component of the process. With the exception of sodium, all the metals that were identified in the study were at very low concentrations close to the analytical reporting limits. The concentrations of metals in Table 8 are extremely low.

Total Organic Carbon Analysis: TOC was measured to determine whether the test solution extracted organic compounds from the contact layer of the test bag/bottle. The results are tabulated in Table 9.

The data in Table 8 suggest that

overall the TOC did increase with increased exposure time. The TOC analysis also suggests that the Vendor B bags have higher extractables than the bottles. Both observations were verified in the detailed organic compounds analysis.

Analysis of Organic Compounds (Volatiles, Semivolatiles, and Nonvolatiles): The analytical methods used in the study are appropriate for identification of all Class I, Class II, and Class III compounds listed in the ICH guidelines.

As described in the introduction, the organic compounds detected in this study were compared with the list of Class I, Class II, and Class III ICH compounds listed in References 7 and 8. This study detected no Class I compounds. The Class II compounds detected in this study were 2-hexanone (CAS 591-78-6) and formamide (CAS 75-12-7). The Class III compounds detected in this study were 2-butanone (CAS 78-93-3), acetone (CAS 67-64-1), 1-pentanol (CAS 71-41-0), and 2-propanol (CAS 67-63-0.)

The sum of the highest concentrations of Class II solvents was used for calculating the worst-case dosage estimate for Class II ICH compounds. Also, the lowest ICH-permitted daily exposure limit of the Class II solvents identified in the study was selected for the calculations. For the worst-case dosage estimate for Class III ICH compounds, the sum of concentrations of the highest extracting test bag/solution combination was selected as the worst-case extractor. These steps are consistent with the assumptions of the worst-case model.

For Class II compounds, the highest observed concentration of 2-hexanone in the study was 96 µg/mL, and the highest observed concentration of formamide was 8 µg/mL. The sum of these concentrations form the highest concentration of Class II compounds observed in the study, 104 µg/mL. The ICH PDE of 2-hexanone is 0.5 mg/day and 2.2 mg/day for formamide. The lower concentration limit of 0.5 mg/day was selected for the worst-case dosage calculations.

The maximum amount of Class II

extractables that could be carried into a batch of drug product in production was determined to be 28,675 µg (approximately 28.7 mg/BDS batch). This is 0.040% of the PDE limit of 0.5 mg/day listed in the ICH guideline, far below the established limit.

For Class III compounds, the highest extracting test bag/solution combination was determined to be 1 M sodium carbonate in a Vendor B, which had a maximum total extractables concentration of 5767 µg/mL. This was selected as the worst-case extractor. The ICH PDE for Class III compounds is 50 mg/day.

The maximum amount of Class III extractables that could be carried into a batch of drug product in production was determined to be 1566 mg. This is 0.022% of the PDE limit of 50 mg/day listed in the ICH guideline — well below the established limit.

Bags from both Vendor A and Vendor B were used in the production facility. In this study, the Vendor B bags consistently show higher extractables than Vendor A bags. However, because the extractables from Vendor B bags are still well below the limits for Class II and Class III compounds, both Vendor A and Vendor B bags can be used interchangeably in the manufacturing process. The study has also shown that 1-L bags or 500-mL PETG bottles can be used for inoculum media.

One concern with material compatibility was observed during the study. A total concentration of 2.8 g/L of 1,4-cyclohexanedimethanol was extracted from the PETG test bottle into the 2 M sodium hydroxide test solution at the 30-day accelerated time point. This result does not affect the extractables study because no basic solutions are currently stored in PETG bottles. However, because of this compatibility issue, basic solutions should not be stored in PETG bottles unless further tests demonstrate material compatibility.

WFI was used to prepare the aqueous solutions used in this study. This choice did result in a significant number of false positives because WFI is exposed to plastics during its manufacture (for instance, filters and

Table 7: Results of conductivity measurement during the extractable study

Test Material and Test Solution	Samples at Accelerated Aging Conditions (40 °C)		
	Conductivity (mS/cm at 25 °C)		
	Day 0	Day 14	Day 30
Vendor B, WFI	<0.01	0.06	0.01
PETG bottle, WFI	<0.01	<0.01	<0.01
Polypropylene bottle, WFI	<0.01	<0.01	<0.01
Control, WFI	0.05	<0.01	<0.01
Vendor B, 2 M sodium hydroxide	>141	>141	>141
PETG Bottle, 2 M sodium hydroxide	>141	>141	>141
Polypropylene Bottle, 2 M sodium hydroxide	>141	>141	>141
Control, 2 M sodium hydroxide	>141	>141	>141
Vendor B, 1 M hydrochloric acid	>141	>141	>141
PETG Bottle, 1 M hydrochloric acid	>141	>141	>141
Polypropylene Bottle, 1 M hydrochloric acid	>141	>141	>141
Control, 1 M hydrochloric acid	>141	>141	>141
Vendor A, 1 M sodium carbonate	69	83	69
Vendor B, 1 M sodium carbonate	70	79	79
Control, 1 M sodium carbonate	69	79	79
Polypropylene bottle, autoclaved WFI	<0.01	<0.01	<0.01
Control, autoclaved WFI	<0.01	<0.01	<0.01
Test Material and Test Solution	Samples at Normal Aging Conditions (25 °C)		
	Conductivity (mS/cm at 25 °C)		
	Day 0	Day 34	Day 72
PETG bottle, 95% ethanol	<0.01	<0.01	<0.01
Polypropylene bottle, 95% ethanol	<0.01	<0.01	<0.01
Control bottle, 95% ethanol	<0.01	<0.01	<0.01

filter cartridges are made of plastics). These false positives were included in the worst-case dosage estimates. This is consistent with the worst-case model that was adopted for the study.

With regard to the issue of extractables, the maximum storage time for the solutions in these containers was determined to be 72 days at 2–27 °C based on the above studies.

AN EXAMPLE VALIDATION STRATEGY

Validation of storage of solutions in BPCs of various sizes was performed to demonstrate microbiological control over the duration of the storage. Small-scale characterization data from process development in support of storage of media solutions in bags

demonstrated that the storage did not affect cellular performance. The validation was performed on biobags from 10 to 750 L and 2-L bottles. The solutions used for storage included cell culture media, calf sera, process solutions used in the upstream process, and buffer solutions. Media solutions known to promote growth were used as a worst-case solution.

The BPCs were categorized into three types: “pillow” type, two-dimensional biobags (10 L), and regular three dimensional biobags (50 L to 750 L) and bottles (2 L). From each category, the largest capacity biobag or bottle was chosen as the representative worst case based on the assessment that the larger the exposed surface area to environment, the

Table 8: Results of metals analysis for the extractable study

Test Material and Test Solution	Metal and Concentration			
	Day 0	Day 14	Day 30	Day 72
Vendor A Sodium carbonate	ND*	ND	0.0063 mg/L Ag 0.012 mg/L Cr	
Vendor B Water for Injection (WFI)	ND	ND	2.1 mg/L Na	
2 M sodium hydroxide	ND	ND	ND	
1 M hydrochloric acid	ND	ND	ND	
1 M sodium carbonate	ND	ND	0.012 mg/L Cr	
PP bottles WFI	ND	ND	ND	
2 M sodium hydroxide	ND	ND	ND	
1 M hydrochloric acid	ND	ND	ND	
95% ethanol/5% water	2.0 mg/L Na		0.010 mg/L Co	ND
Autoclaved WFI	ND	ND	0.12 mg/L Fe	
PETG bottles WFI	ND	ND	ND	
2 M sodium hydroxide	ND	0.018 mg/L Mn	0.026 mg/L Mn	
1 M hydrochloric acid	2.0 mg/L Na	2.0 mg/L Na	2.0 mg/L Na 0.26 mg/L Fe	
95% ethanol/5% water	0.053 mg/L Pb		0.012 mg/L Co	0.026 mg/L Zn

*ND = No metals detected above the reporting limit.

Table 9: Results of total organic carbon analysis for the extractable study

Test Material and Test Solution	Total Organic Carbon (mg/L)		
	Day 0	Day 14	Day 30
Vendor B, WFI	0.49	7.8	9.5
PETG bottle, WFI	0.3	0.42	0.54
Polypropylene bottle, WFI	1.1	0.43	0.49
Control, WFI	0.29	0.68	0.43
Polypropylene bottle, Autoclaved WFI	0.9	0.76	0.94
Control, autoclaved WFI	0.43	0.32	0.34

higher the risk of microbial ingress. Table 1 shows the list of solutions stored in BPCs at an Amgen facility and the storage conditions. The temperatures of storage, depending on the solution, varied from 2 to 8 °C to ambient conditions. The validation was performed at ambient conditions as a worst case.

A family/worst-case approach was taken for these validation runs with three containers of each type put on validation and periodic samples taken for microbial content testing. The acceptance criteria for microbial

content in these samples were based on the in-process acceptance criteria for the downstream.

Validation Results: With the in-process acceptance criteria as shown in Table 2, all results were found to be within the acceptance criteria of the validation study. Table 11 shows the durations of the study using various containers. The recommended hold time for all BPCs was 75 days.

AN EASY JUSTIFICATION

Based on the test results, the use of the BPCs tested in this study can

easily be justified for their use in manufacturing. The worst-case dosage estimate, based on ICH guidelines, shows that no Class I solvents were found in the extractables study. The sum of the highest possible amount of Class II extractables for one dose of drug product was calculated to be only 0.04% of the ICH PDE limit of 0.5 mg per day. The sum of the highest possible amount of Class III extractables in one dose of drug product was found to be only 0.022% of the ICH PDE limit of 50 mg/day.

The results of the extractables study show that implementation of biobags in this manufacturing process does not affect the drug product by adding extractables above the permitted limits set by the ICH. The study also shows that both Vendors A and B provide biobags of similar materials that are both suited to this process.

The study also provides a basis to show comparability between the two vendors' products. Based on the levels of extractables, bags from both vendors may be used interchangeably throughout production. The study also shows that the PETG bottles are compatible with the production process.

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FOR FURTHER READING

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Table 10: Container categories and worst-case scenarios

Container Category	Container Volumes (L)	Worst-Case Container	Vendor	Worst-Case Storage Conditions
Pillow bags	5, 10	10 L	A, B	Ambient, Class 100,000
Regular bags	50, 100, 200, 250, 500, 750	750 L	A, B	Ambient, Class 100,000
PETG bottles	0.5, 2	2 L	C	Ambient, Class 100,000

Table 11: Validation hold times

Container	Run #	Run Start Date	Last Passing Sample Date	Study Duration (Days)	Shortest Duration (Days)
Vendor B 10-L pillow bags	1	07-12-04	09-28-04	77	77
	2	07-12-04	09-28-04	77	
	3	07-12-04	09-28-04	77	
Vendor A 10-L pillow bags	1	07-12-04	09-28-04	77	77
	2	07-12-04	09-30-04	79	
	3	07-12-04	09-30-04	77	
Vendor B 750-L regular biobags	1	07-14-04	09-28-04	75	75
	2	07-14-04	09-28-04	75	
	3	07-14-04	09-28-04	75	
Vendor A 750-L regular biobags	1	07-12-04	09-28-04	77	77
	2	07-12-04	09-28-04	77	
	3	07-12-04	09-28-04	77	
2-L Vendor C bottles	1	07-12-04	09-28-04	77	77
	2	07-12-04	09-28-04	77	
	3	07-12-04	09-28-04	77	