

DRUG CONTAINER CLOSURE TESTING: IMPACT OF EXTRACTION AND ANALYSIS METHODOLOGY ON METAL CONTAMINANT QUANTIFICATION

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Regulatory Agencies have clearly established the need to identify and control harmful chemicals which may leach from container closure systems into the final packaged drug product. While inorganic metal additives are necessary components in the manufacture of the container closure polymers, these same compounds may be considered adulterants in the final drug product. Potential toxicity of some metals at extremely low levels is well established.

The United States Pharmacopeia (USP) details colorimetric methods for detecting heavy metal contaminants in elastomers, glass and polymers. Unfortunately, these methods are well known to be inadequate for identification and quantification of these same contaminants at the trace levels where they exhibit potential toxicity. Consider the method for detecting heavy metals in plastics:

USP <661> COLORIMETRIC METHOD:

1. 20 mL of sample preparation extract adjusted to a pH between 3.0 and 4.0 and volume adjusted to 35 mL.
2. 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer are added, diluted to 50 mL with water and mixed.
3. Any brown color formation in 10 minutes exceeding that of a Standard Lead solution tube indicates heavy metals are present.

This method balances on the intensity of the color of sulfide precipitation in an extract. It is non-specific, insensitive, time consuming and labor intensive. The level of detection for lead in this colorimetric test, about 10 ppm, is much lower than current data on lead toxicity. A USP Ad-

visory Panel recommended an oral limit for lead of 1 µg/g and a parenteral limit of 0.1 µg/g. Also consider the FDA lead specification of 5 ppb for bottled drinking water. A small amount of lead in a plastic bottle closure leached into an aqueous product would readily exceed this limit.

Of course lead is not the only metal of concern which may leach into a drug product from its container closure. USP Proposed Chapter <232> Elemental Impurities – Limits base assessment of metals for toxicological risk of elemental classifications. Class 1 Elemental Impurities have high levels of toxicity and should not be present in drug product. It is conceivable that container/closures containing these elements may potentially contribute impurities to the drug product. USP recommends restricting levels as shown in Table 1.

Our laboratory conducted a series of experiments to measure the impact of extraction, solvent, and detection techniques on Class 1 and Class 2 element recovery. Four types of container closures were randomly selected and tested by requisite USP techniques. All samples met USP criteria. Additional samples were extracted with four different solvents by three different vigorous extraction methods. The resulting extract was analyzed by Atomic Adsorption (AA), ICP OES, and ICP MS.

TABLE 1: USP <232> CLASS 1 ELEMENTS

ELEMENT	COMPONENT LIMIT (ppm)	ORAL DAILY DOSE (µg/day)	PARENTERAL COMPONENT LIMIT (ppm)	PARENTERAL DAILY DOSE (µg/day)
Arsenic	1.5	15	0.15	1.5
Cadmium	0.5	5	0.05	0.5
Lead	1.0	10	0.1	1.0
Mercury	1.5	15	0.15	1.5

METHODOLOGY:

Samples:

Two containers and two closures spanning the spectrum from low leachable risk (solid oral dosage forms) to high leachable risk (parenteral) were tested. Container closures span typical materials.

1. High density polyethylene bottle: 16 oz white screw cap
2. Polypropylene cap: white screw cap to 2000 cc bottle
3. Grey elastomeric closures: 10 x 20 mm
4. 2 mL glass vials

Solvents:

Four polar solvents with varying pH and oxidative ability were utilized to extract the metals.

1. Water for Injection (WFI) (Baxter C795252, exp 02/11)
2. 0.1 Normal HCl (Ricca Lot 2008191, exp. 07/12)
3. 0.1 Normal NaOH (Ricca Lot 2908403, exp. 08/11)
4. 1.0 % H₂O₂ (Loreo Lot 091409, exp. 09/24/15).

Extraction Techniques:

Plastic and stoppers were cut to pieces approximately 1 mm thickness. Glass was crushed to small uniform pieces. Approximately 25 gm of cut/crushed container closure pieces were added to 250 mL of each solvent listed above, and then extracted according to the following techniques:

1. Heat at 70°C for 24 hours.
2. Autoclaved at 121°C for 1 hour
3. Refluxed in Soxhlet Apparatus for 24 hours

Analytical Techniques:

Resulting extracts were tested for the presence of metals by each of four methods:

1. **AA:** Resulting extracts were tested with a Spectro Atomic Absorption Apparatus. A standard curve was developed with reference standards. With the exception of mercury analysis, samples were atomized by flame. Mercury was atomized by cold vapor.
2. **ICP OES:** Resulting extracts were tested with a Spectro ICP OES Apparatus using standards. Inductively coupled plasma produced excited atoms and ions that emit elector magnetic radiance at a wavelength characteristic of that element.

3. **ICP MS:** Resulting extracts were tested with a Varian ICP MS. Inductively coupled plasma produces excited atoms and ions which are separated based on mass-to-charge ratio. Our instrument is sensitive to parts per trillion level, but we reported results only to parts per billion.

In all analytical techniques, the results were corrected for solvent controls. Solvent controls were run through the same extraction techniques prior to analysis.

Elemental Analysis Focus:

This study primarily focused on Class 1 and Class 2 Elemental Impurities as defined by proposed USP <232>.

Class 2 Elemental Impurities should be limited in drug products because of their inherent toxicities. USP recommended levels for the compounds screened in this study are presented in Table 2.

TABLE 2: USP PROPOSED CLASS 2 ELEMENT

ELEMENT	COMPONENT LIMIT (ppm)	ORAL DAILY DOSE (µg/day)	PARENTERAL COMPONENT LIMIT (ppm)	PARENTERAL DAILY DOSE (µg/day)
Copper	250	2500	25	250

TABLE 3: ZINC CONCENTRATION IN ELASTOMERIC STOPPERS IN VARIOUS SOLVENTS

SOLVENT	TEMPERATURE	USP <661> (ppm)	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
Water	N/A	negative	N/A	N/A	N/A
1% H ₂ O ₂	70°C	N/A	<0.1	<0.1	0.047
WFI	70°C	N/A	0.12	<0.1	0.143
0.1 N HCl	70°C	N/A	<0.1	<0.1	0.022
0.1 N NaOH	70°C	N/A	<0.1	<0.1	0.098
WFI	121°C	N/A	<0.1	<0.1	0.035
0.1 N HCl	121°C	N/A	0.1	0.2	0.138
0.1 N NaOH	121°C	N/A	<0.1	<0.1	0.041
1% H ₂ O ₂	121°C	N/A	0.1	<0.1	0.099
WFI	Soxhlet	N/A	0.2	0.2	0.16
1% H ₂ O ₂	Soxhlet	N/A	0.1	<0.1	0.094
0.1 N HCl	Soxhlet	N/A	0.3	0.4	0.272
0.1 N NaOH	Soxhlet	N/A	0.7	0.7	0.272

TABLE 4: ZINC CONCENTRATION IN GLASS VIALS

SOLVENT	TEMPERATURE	USP <661> (ppm)	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
Water	N/A	negative	N/A	N/A	N/A
WFI	70°C	N/A	<0.1	<0.1	0.010
0.1 N HCl	70°C	N/A	<0.1	<0.1	0.050
0.1 N NaOH	70°C	N/A	0.4	0.7	0.314
1% H ₂ O ₂	70°C	N/A	<0.1	<0.1	0.018
WFI	121°C	N/A	<0.1	<0.1	0.016
0.1 N HCl	121°C	N/A	0.1	0.1	0.080
0.1 N NaOH	121°C	N/A	0.8	0.8	0.327
1% H ₂ O ₂	121°C	N/A	<0.1	<0.1	0.022
WFI	Soxhlet	N/A	<0.1	<0.1	0.071
0.1 N HCl	Soxhlet	N/A	0.3	0.2	0.207
0.1 N NaOH	Soxhlet	N/A	0.7	0.3	0.249
1% H ₂ O ₂	Soxhlet	N/A	0.1	<0.1	0.022

TABLE 5: COPPER IN HDPE BOTTLES

SOLVENT	TEMPERATURE	USP <661> (ppm)	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
Water	N/A	negative	N/A	N/A	N/A
WFI	70°C	negative	<0.1	<0.1	0.009
0.1 N HCl	70°C	negative	<0.1	<0.1	0.003
0.1 N NaOH	70°C	negative	<0.1	<0.1	0.161
1% H ₂ O ₂	70°C	negative	<0.1	<0.1	0.005
WFI	121°C	negative	<0.1	<0.1	0.002
0.1 N HCl	121°C	negative	<0.1	<0.1	0.01
0.1 N NaOH	121°C	negative	<0.1	<0.1	0.117
1% H ₂ O ₂	121°C	negative	<0.1	<0.1	0.005
WFI	Soxhlet	negative	<0.1	<0.1	0.041
0.1 N HCl	Soxhlet	negative	0.6	0.6	0.484
0.1 N NaOH	Soxhlet	negative	0.3	<0.1	0.222
1% H ₂ O ₂	Soxhlet	negative	0.1	0.2	0.17

Extractants were screened for the following metals:

1. Arsenic: Class 1 Compound
2. Cadmium: Oxides of cadmium are sometimes used as colorants
3. Mercury: Class 1 Compound
4. Lead: Class 1 Compound, sometimes added to enhance aging performance
5. Iron: Oxides of iron sometimes used as colorant
6. Copper: Class 2 Compound, content should be limited
7. Zinc: Commonly used as an accelerator, lubricant, and heat stabilizer

1,536 data points were collected. We present only those data which show detection of element by at least one of the methods in our matrix.

RESULTS

Zinc concentration in elastomeric closures varies from undetected or 22 ppb to 700 ppb dependent upon the solvent, extraction method, and detection method chosen. This is a variance of 350%. Extraction of elastomeric closure in a basic solution with Soxhlet apparatus gives the highest concentration of zinc. (Table 3)

Zinc concentration in glass vials varied from undetected or 10 ppb to 800 ppb. The most effective means of extracting zinc from glass vials is with acid or base at autoclave temperatures or with Soxhlet apparatus. (Table 4)

Copper was present in HDPE bottles in trace quantities, but was best recovered with Soxhlet extraction with acid, base, and oxidative solvents. (Table 5)

Trace levels of iron were detected in the polypropylene closures, but only when extracted with Water for Injection or 0.1 N HCl, and only under high heat extraction. (Table 6)

TABLE 6: IRON IN POLYPROPYLENE CLOSURES

SOLVENT	TEMPERATURE	USP <661> (ppm)	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
Water	N/A	Negative	N/A	N/A	N/A
WFI	70°C	N/A	<0.1	<0.1	<0.001
0.1 N HCl	70°C	N/A	<0.1	<0.1	0.124
0.1 N NaOH	70°C	N/A	<0.1	0.1	0.077
1% H ₂ O ₂	70°C	N/A	<0.1	<0.1	0.01
WFI	121°C	N/A	<0.1	0.3	0.297
0.1 N HCl	121°C	N/A	1.1	0.5	0.499
0.1 N NaOH	121°C	N/A	<0.1	<0.1	0.026
1% H ₂ O ₂	121°C	N/A	<0.1	<0.1	<0.100
WFI	Soxhlet	N/A	<0.1	<0.1	0.016
0.1 N HCl	Soxhlet	N/A	<0.1	<0.1	<0.001
0.1 N NaOH	Soxhlet	N/A	<0.1	<0.1	<0.001
1% H ₂ O ₂	Soxhlet	N/A	<0.1	<0.1	<0.001

TABLE 7: MERCURY IN ELASTOMERIC CLOSURES

SOLVENT	TEMPERATURE	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
WFI	70°C	0.204	0.5	0.12
0.1 N HCl	70°C	0.125	<0.1	0.03
0.1 N NaOH	70°C	0.001	<0.1	0.027
1% H ₂ O ₂	70°C	0.002	<0.1	0.002
WFI	121°C	<0.001	<0.1	<0.001
0.1 N HCl	121°C	0.006	<0.1	<0.001
0.1 N NaOH	121°C	0.001	<0.1	<0.001
1% H ₂ O ₂	121°C	<0.001	<0.1	<0.001
WFI	Soxhlet	<0.001	<0.1	<0.001
0.1 N HCl	Soxhlet	<0.001	<0.1	<0.001
0.1 N NaOH	Soxhlet	<0.001	<0.1	<0.001
1% H ₂ O ₂	Soxhlet	<0.001	<0.1	<0.001

TABLE 8: LEAD CONTENT IN POLYPROPYLENE CLOSURE EXTRACTED AT 70°C FOR ONE HOUR

SOLVENT	TEMPERATURE	USP <661> (ppm)	AA (ppm)	ICP OES (ppm)	ICP MS (ppm)
Water	N/A	negative	N/A	N/A	N/A
WFI	70°C	N/A	1.7	0.5	1.7

Trace levels of mercury were detected in stoppers when extracted at lowest temperature over a 24 hour period. (Table 7) Lead concentration in polypropylene closure of 1.7 ppm was found in a Water For Injection (WFI) extract of a polypropylene closure at 70°C. Lead was not found in other solvents nor by USP Heavy Metal Test. (Table 8)

CONCLUSIONS

Our data demonstrate:

1. There is a high degree of variability in metal content of test container closures depending on method of extraction and detection.
2. In all cases, instrumental analysis is significantly more sensitive than USP <661> Colorimetric method.
3. Mercury is better captured at lower extraction temperatures.
4. ICP MS is most sensitive and reliable detection technique.

When performing extractable studies, it is best practice to vary extraction techniques and solvents to maximize recovery of metals. SGS Life Science Services offers its expertise to assist clients with analytical method development for container testing and elemental extractables specific to their products.

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