NEW TESTS FOR ELASTOMERIC CLOSURES: USP <381>

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It's an event that repeats itself hundred's of thousand's of times a day. A stainless steel needle pierces an elastomeric closure clear through to the other side, breaching the barrier that protects an inner sterile environment from the outer, impure world. The closure material yields just enough for the needle to easily penetrate, while simultaneously closing tightly around the needle without fragmenting, splintering, or crumbling.

After the syringe withdraws drug content from the vial, the needle is removed and the closure material reseals, again forming an impervious barrier to the outside world. For many weeks prior, this closure has already performed a miracle task, forming a tight seal in the vial and preventing entry of outer microbes and chemicals. Through shipping, storage, temperature and pressure fluctuations, it has never leached lubricants, colorants, or other additives into the drug product. The "simple" vial stopper is really a great example of high end pharmaceutical technology.

But what tests are performed to ensure this miracle product performs as advertised? The minimal testing expectations for such closures is outlined in USP <381> Elastomeric Closures for Injection. Prior to May 1, 2009, this monograph required only a handful of Biological and Physicochemical tests. But now the testing requirements have greatly increased to better reflect the important role that this component plays. If the pharmaceutical manufacturer has not confirmed performance to these minimal biological, physicochemical, and functional test requirements in the new enhanced guideline, it is not in compliance with current guidelines and has not confirmed that the closure is in fact safe to use. Definition of testing responsibility is an important distinction made in this revised General Test Chapter – "Elastomeric closures shall conform to biological, physicochemical, and functionality requirements both as they are shipped by the closure supplier to the injectable product manufacturer (the end user) and in their final ready-to-use state by the end user."[1] This article highlights changes in the new chapter and briefly describes the tests prescribed by it.

SECTION	TEST	USP 31	USP 32
BIOLOGICAL TEST	Safety	USP <87> *In vitro* USP <88> *In vivo*	USP <87> *In vitro* USP <88> *In vivo*
PHYSICOCHEMICAL TESTS	Turbidity	USP <851>	USP <851> or Visual
	Reducing Agents	Purified Water Extract	Purified Water extract plus permanganate
	Heavy Metals	USP <231>	USP <231>
	Extractable Zinc	N/A	USP <851>
	pH Impact	pH of extract versus blank	Bromythymol blue titration of extract versus blank
	Total Extractables	Gravimetric analysis of dried extract	N/A
	Absorbance	N/A	Absorbance of extract at 220 – 360 nm
	Ammonium	N/A	Colorimetric test
	Volatile Sulfides	N/A	Colorimetric test
	Colorants	N/A	Visual Comparison
	Identification	N/A	FTIR, other
FUNCTIONAL TESTS	Penetrability	N/A	Force of no greater than 10 N
	Fragmentation	N/A	Visual observation of filtrate
	Self Sealing	N/A	Visual observation after vacuum soak in methylene blue

TABLE 1: SUMMARY OF USP <381> TEST CHANGES AFTER MAY 1, 2009

CHANGES

Table 1 outlines the changes to the new USP monograph. The monograph outlines three sections of Test Procedures:

1. Physicochemical Tests: chemical analysis of closure extracts
2. Biological Tests: observation of effects of closure extracts on biological systems
3. Functional Tests: observation of physical performance of full closure.

While there were no substantive changes to the Biological Tests, additional procedures were added to the Physicochemical Tests, and the Functional Tests category is completely new. In addition, although the chapter does not outline specific procedures, USP <381> now suggests the end user must confirm container closure integrity, freedom from particulates, endotoxin, and leachables.

EXTRACTION

Extraction principles are essentially unchanged in the new test chapter. Uncut closures are washed, suspended in purified water, and extracted in an autoclave. However, volumes of water and duration of extraction have changed.

PHYSICOCHEMICAL TEST

Most modern elastomers are of synthetic origin and as a result, can bring in many unintended contaminates to the product. Metals, zinc in particular, are used as curing agents in production of rubbers. Colorants are added to enhance consumer appeal. Sulfur might be added as a curing agent. Antioxidants, vulcanizers, peroxides, accelerators, coupling agents, strength modifiers, release agents and lubricants are examples of other potential contaminants. The revised USP <381> chapter greatly enhances the few gravimetric tests in the old General Test Chapter. For example, an AA or ICP will now be required to detect the presence of zinc. Now, specific contaminants are screened (ammonia, sulfide, colorants) and the polymer itself should be identified by IR. But in no way are these tests alternatives for a full extractable and leachable study. In fact, the new General Test Chapter underscores that it is the responsibility of the end user to assess testing needs which might be beyond the scope of this chapter. The handful of colorimetric tests listed in the new USP <381> are an improvement over the single gravimetric test in the old chapter, but is no substitute for a full extraction and identification by mass spectrophotometry or other technologies. For more information on Extractable and Leachables studies, please consult an SGS technical article on this topic.

BIOLOGICAL TESTS

Two stages of tests are indicated to screen for potential biological impact of elastomer extracts. The first stage is described as USP <87> Biological Reactivity Tests, *in vitro*. Essentially, an extract of the sample or a surface of the sample itself is placed in contact with a healthy monolayer of cultured L929 mammalian fibroblasts for several days. At the end of the test period, the cells are examined for cytopathic effects. Any rounding, nucleosis, or cell death indicates the elastomer will leach compounds that could be harmful. Of course, a monolayer of immortalized cells in direct contact with the test sample or extract of the test sample for several days is not exactly analogous to a real world dosing. But it is a quick and inexpensive screen for biological impact. Materials that meet the requirements of the *in vitro* test are not required to undergo *in vivo* testing.

FUNCTIONAL TESTS

Introduced in this revision for the first time, USP <381> now prescribes tests to confirm that the closure is easily penetrated, does not fragment, and self seals after puncture. A lubricated long bevel hypodermic needle is used, and forces and pressures are carefully defined. Self sealing capacity in particular is important in confirming the closure protects the drug product from contamination after multiple samplings. Closures crimped to vials are penetrated by a beveled hypodermic needle under controlled conditions, and then immersed in a dye solution under vacuum. Atmospheric pressure is returned, vials are recovered, and internal solutions are examined for dye infiltration. Although this test is a useful start, it doesn't detail the validation process necessary to confirm finished product does not interfere with color changes as a result of dye penetration. The test can also be modified to screen for microbial ingression. SGS offers complete Container Closure Integrity tests to confirm vial seals will prevent microbial and other contamination after multiple injections.

CONCLUSION

As of May 1, 2009 USP <381> enhances the tests necessary to confirm the safety of elastomeric closures. Biological Tests are again prescribed, physicochemical tests are improved, and functional tests are added. SGS is your one source contract research organization, offering all of these tests under one roof. Our pharmaceutical packaging group performs extraction, tissue culture, AA or ICP, and functionality tests. No tests are subcontracted.

But the chapter is still only a first step, a screen, and the new language in the General Test Chapter clearly indicates as much. SGS can help determine if your product also needs Leachable and Extractable Testing, Container Closure Integrity testing, shipping simulation studies, or other non-compendial investigations. Contact your local SGS facility for more information.

Reference: [1] USP 32 <381> Elastomeric Closures for Injections

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