Assigned AAPS Poster Number
T3129

Extractables and Leachables: Where do Surfactants Belong?

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BACKGROUND

Rational:

Every new drug application is expected to include some form of extractables profiling and leachables evaluation for the container closure or manufacturing components at highest risk and in closest contact with the drug.

Regulatory Guidance:

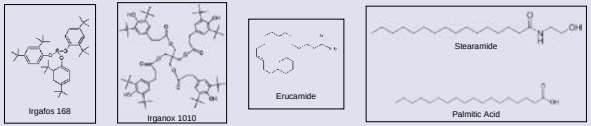
- *US Title 21, Code of Federal Regulations (CFR) Part 211.94 (a) "Drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug beyond the official or established requirements."*
- *US Title 21, Code of Federal Regulations (CFR) Part 211.65 (a) "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."*

Extractables and Leachables:

- **Extractables -** compounds that can be extracted from a component under *exaggerated* conditions such as in the presence of harsh solvents and/or at elevated temperatures.
- **Leachables -** compounds that leach into the drug product formulation or in-process matrices from the component as a result of direct contact with the formulation under *normal* conditions or accelerated storage conditions.

Why Worry About Extractables and Leachables:

- May increase the impurity level of drug product to an unacceptable level
- May react with one or more of the drug product components
- May increase toxicity of drug product



CASE STUDY

Introduction:

In order to demonstrate that a container closure system or a bioprocessing component is suitable for its intended use, the components typically undergo an initial extractables screening. The design of the extraction experiment should appropriately exaggerate the conditions of real time use without breaking down or degrading the polymeric component under test. For bioprocessing components, initial extractables screening involves filling or immersing the component in a variety of model solvents. The components are incubated in the solvent for a predetermined length of time at an elevated temperature, such as 40°C to 60°C for several days, weeks, or even months.

Many extractables studies have employed model solvents that are comprised of the same excipients that are present in the process buffers, drug substances, or final drug product formulations. These excipients often include surfactants, which are common ingredients in the formulations of biologics. However, surfactants pose major chromatographic interferences when screening for non-volatile organic compounds by high-performance liquid chromatography/mass spectrometry (HPLC/MS). The detection of extractable compounds may be masked by co-eluting surfactant peaks, and high concentrations of surfactants can contaminate the HPLC/MS system. Dilution is not always a viable solution because sensitivity can be greatly affected.

Question:

Is the use of formulation buffers, containing excipients such as surfactants, a good strategy for generating initial extractables profiles?

Methods:

Polytetrafluoroethylene (PTFE) lined polypropylene (PP) caps were extracted at 40°C/Ambient Relative Humidity for seven days with 25 mL of each of the following surfactants: 1% Triton X-100, 0.1% Polysorbate 80 (PS 80), and 0.1% Polysorbate 20 (PS 20). The same caps were also extracted with the following two alternative solvents: 60% IPA and 15% Ethylene glycol monobutyl ether (EGMB). The resulting extracts were tested by gradient HPLC using an Agilent 6500 series LC/MS-Time of Flight (TOF) equipped with a multimode source (electrospray and atmospheric pressure chemical ionization) using positive ionization (MMP). The caps were chosen for this experiment due to the presence of known additives that are easily tracked during extractables screening. The compounds that were targeted in this experiment include: Irgafos 168, Irgafos 168 phosphate, ethylene bis heptadecanamide, Irganox 1010, and erucamide.

Data:

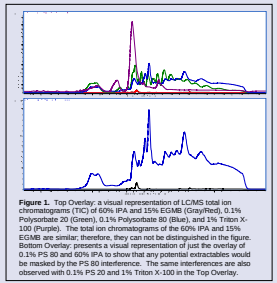


Figure 1. Top Overlay: a visual representation of LC/MS total ion chromatograms (TIC) of 60% IPA and 15% EGMB (Gray/Red), 0.1% Polysorbate 20 (Green), 0.1% Polysorbate 80 (Blue), and 1% Triton X-100 (Purple). The total ion chromatograms of the 60% IPA and 15% EGMB are similar; therefore, they can not be distinguished in the figure. Bottom Overlay: presents a visual representation of just the overlay of 0.1% PS 80 and 60% IPA to show that any potential extractables would be masked by the PS 80 interference. The same interferences are also observed with 0.1% PS 20 and 1% Triton X-100 in the Top Overlay.

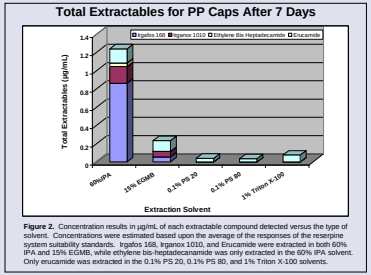


Figure 2. Concentration results in µg/mL of each extractable compound detected versus the type of solvent. Concentrations were estimated based upon the average of the responses of the reserpine system suitability standards. Irgafos 168, Irganox 1010, and Erucamide were extracted in both 60% IPA and 15% EGMB, while ethylene bis-heptadecanamide was only extracted in the 60% IPA solvent. Only erucamide was extracted in the 0.1% PS 20, 0.1% PS 80, and 1% Triton X-100 solvents.

RESULTS

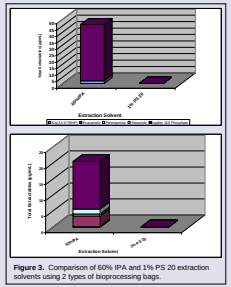


Figure 3. Comparison of 60% IPA and 1% PS 20 extraction solvents using 2 types of bioprocessing bags.

1. In this study, 60% IPA was shown to have the most robust extraction profile extracting compounds that the surfactants could not.
2. These results were comparable to findings from a related study, in which two types of bioprocessing bag films were extracted in various types of solvents (Figure 3).
3. The study also showed that 60% IPA had a greater extraction efficiency compared to the other solvents evaluated including 1% PS 20. In addition to having greater extraction efficiency, 60% IPA was also shown to be free of interferences observed in the sample chromatography of surfactants.
4. The use of extraction solvents that do not pose significant chromatographic interferences is critical so that potential extractable compounds are observed during the extractables screening.

CONCLUSIONS

1. Extractables studies should be done first and separate from leachables studies.
2. Extractable profiles should be generated with model solvents. For extractable screening studies, organic solvents should be used to generate the worst case scenario of extractable compounds.
3. Once these profiles are generated leachables can be targeted with formulation buffers containing excipients such as surfactants in the formulation.
4. Data mining can be done in an extractable study based on the IPA extractables profile.

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