CASE STUDY

EXTRACTABLES & LEACHABLES IN SINGLE-USE SYSTEMS

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BIOPHARMACEUTICAL SAFETY: IDENTIFYING AND QUANTIFYING EXTRACTABLES AND LEACHABLES FROM SINGLE-USE PLASTIC BIOPROCESS BAGS

Contaminants, additives or degradants may leach out from any single-use plastic bioprocess bags used in the preparation, storage and transport of biopharmaceutical liquids, intermediates and final bulk products, causing contamination of biopharmaceutical drug products. Therefore, an important part of the safety studies associated with these products is the identification of any potential extractables and leachables, as they pose a risk to the cell culture process, and potentially even to patients.

Therefore, an important part of safety studies associated with the manufacture and handling of biopharmaceutical products is that any such ‘leachables’ are both identified and quantified. The consequences of the contamination are that they could be toxic to the cells used within the biopharmaceutical production, or potentially have risks to patients either through their direct toxicity, or because of any effects that they may have on the efficacy of biopharmaceutical drug products.

EXTRACTABLES AND LEACHABLES STUDY

Working with Thermo Fisher Scientific, SGS recently carried out an ‘extractables’ study on single-use plastic bioprocess bags using different solvent systems, including acidified water, alkaline water, phosphate-buffered saline (PBS), and mixtures of organic and aqueous solvents. These represented typical pH values, ionic strengths and hydrophobicities of solutions used in biopharmaceutical processing, with a range of analytical techniques being applied to produce a comprehensive profile of the chemical identities and properties of the extractables. These techniques included headspace analyses using gas chromatography–mass spectrometry (GC-MS) and gas chromatography with flame ionization detector (GC-FID) for volatile organic compounds; liquid injection GC-MS and GC-FID for semi-volatile organic compounds; and liquid chromatography–mass spectrometry (LC-MS) and liquid chromatography with ultraviolet detection (LC-UV) analysis for non-volatile organic compounds.

Commercially-available plastic bioprocess bags were used in the tests and extractions were carried out with water at pH = 3, water at pH = 9, PBS, 1:1 isopropyl alcohol (IPA):water mixtures and 1:1 ethanol:water mixtures. In order to promote a leaching effect. Five sets of bags, each set containing one of the five different solutions, were shaken at 50°C for seven days and the extract solutions then analyzed using the various techniques mentioned above.

HEADSPACE ANALYSES

An Agilent 6890N analyzer was used for the headspace GC-MS analysis of volatile organic compounds, giving both qualitative and quantitative results. Cyclohexanone was found to be present in every extract and this was confirmed by liquid-injection GC-MS analysis, thus demonstrating the reliability and completeness of the headspace GC-MS analysis for determining the volatile/semi-volatile organic compound profile of the extract.

Headspace analysis also detected IPA in all of the aqueous extracts and so it was presumed that vapor from the IPA/water extract in the bags could migrate into the aqueous solvents inside the other bags that were inside the same shaker. Following further studies, in which the bags containing aqueous solvents and the ones containing organic solvents were kept apart within the shakers, IPA was not seen to be present in the aqueous extracts. It was also found that the amounts of IPA contaminating the aqueous solutions could be greatly decreased by covering the various bags with aluminum foil, even when the bags were not separated from each other within the shaker.

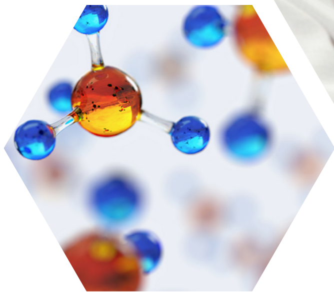

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Figure 1: Ethanol/Water Extract without DCM Extraction

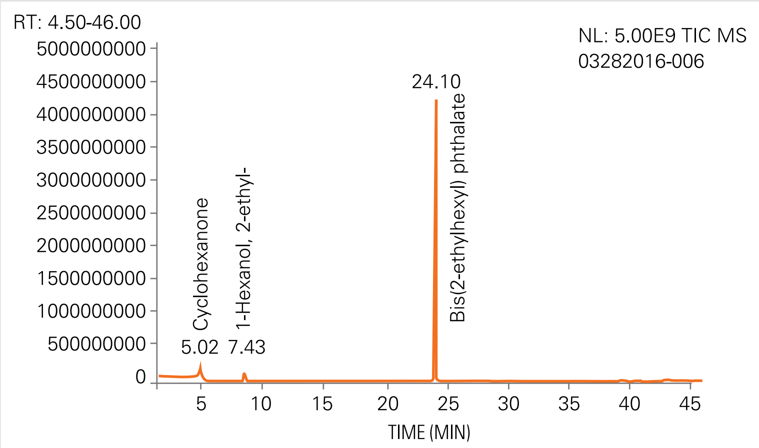


Figure 2: Ethanol/Water Extract with DCM Extraction

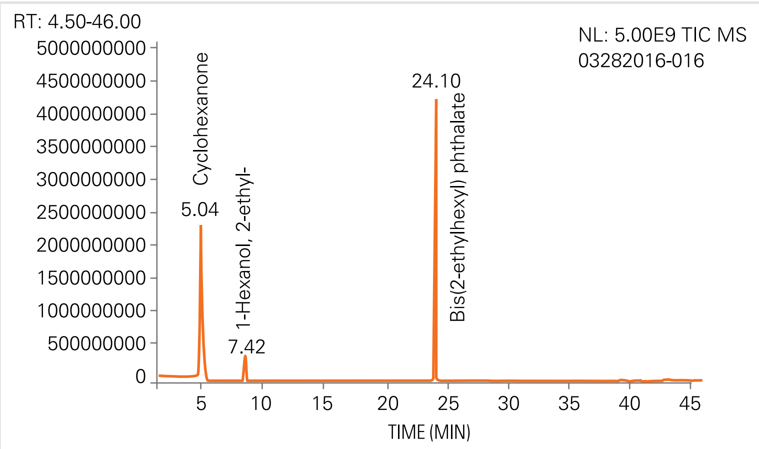


Figure 3: IPA/Water Extract without DCM Extraction

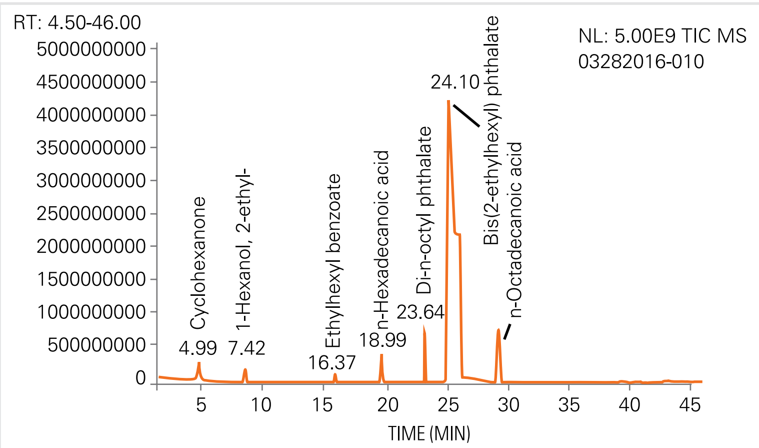
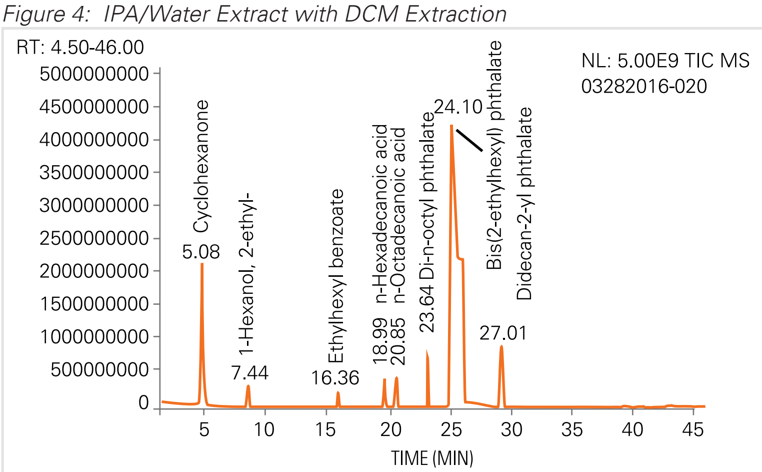


Figure 4: IPA/Water Extract with DCM Extraction



SEMI-VOLATILES: GC-MS ANALYSIS

Semi-volatile organic compounds were identified and quantified by GC-MS analysis with a Thermo Scientific™ ISQ™ LT single-quadrupole system with NIST library, and a Thermo Scientific QExactive™ high-resolution accurate mass (HRAM) GC-MS/MS system was used to help confirm sample identification. In this procedure, it is usual to solvent-exchange aqueous extracts with dichloromethane (DCM) before injection, however emulsification can be a problem for liquid–liquid extraction procedures if there are large amounts of alcohol present in samples. Solvent-exchange effects were therefore studied by injecting alcohol/water (both ethanol and IPA) extracts with and without DCM extraction. The result was a clearer, more identifiable peak shape and better recovery when DCM extraction was used, emulsion and phase separation issues having no effect on the results (see Figs 1-4).

The IPA/water extract had the highest concentration of extractables, mostly comprised of hydrophobic compounds. Bis-(2-ethylhexyl)phthalate (DEHP) was the principal component detected: this compound is the primary plasticizer used in the plastic bags. Most of the other extractables consisted of phthalates, DEHP degradation products and various lubricants.

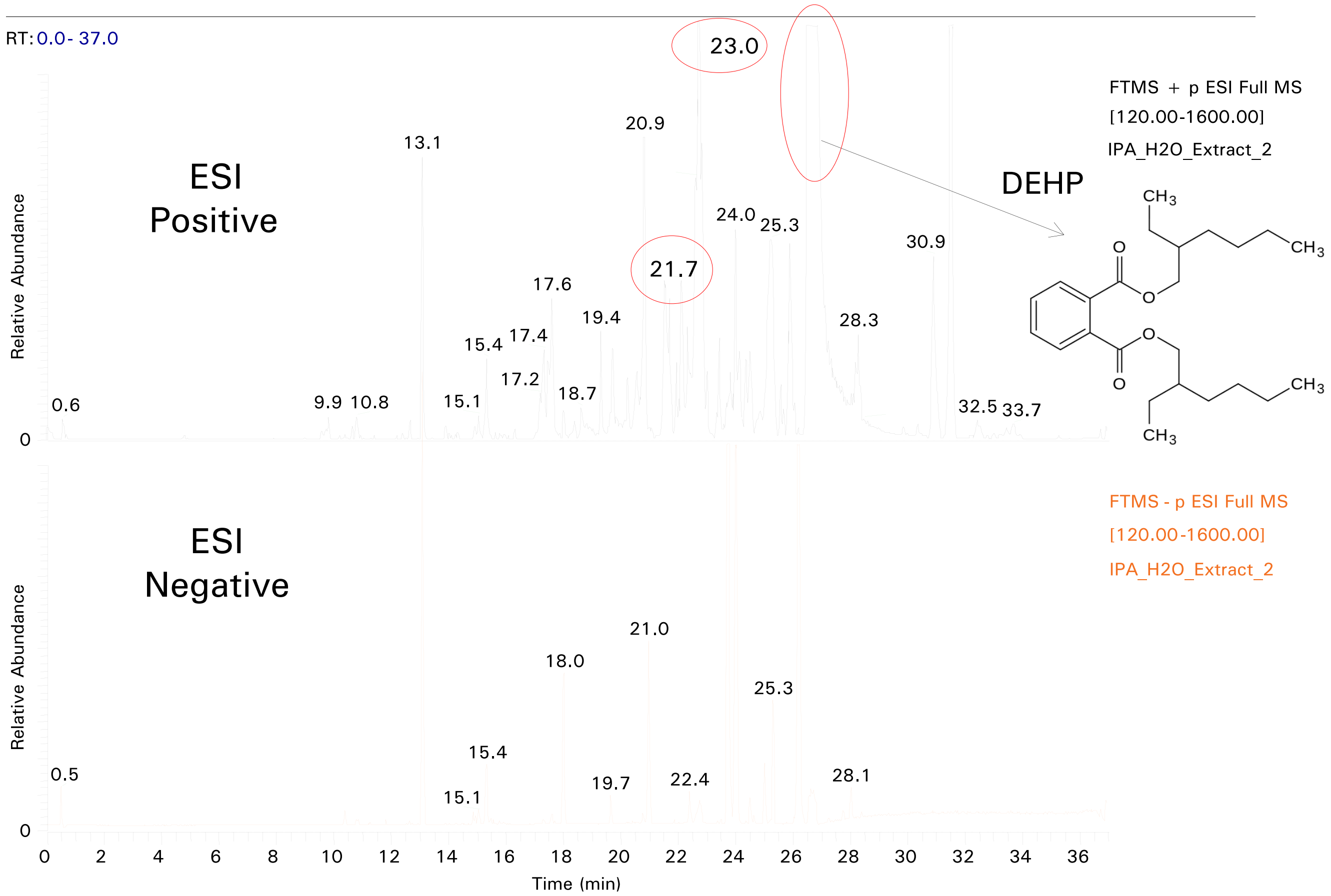
LC-MS FOR NON-VOLATILES

Non-volatile organic compounds were identified and quantified by LC-MS analysis using a Thermo Scientific Q Exactive HRAM LC-MS/MS system. The identification of unknown compounds in this analysis for non-volatiles was more challenging because of the severe lack of standardized spectral libraries: LC-MS spectra vary from vendor to vendor quite significantly. Therefore, in order to aid structure determination, a HRAM full scan and MS/MS data acquisition with polarity switching was employed, ensuring that structurally diverse compounds were detected and a comprehensive extractables profile obtained. Another benefit of using HRAM was an increased analysis throughput.

A total of 20 extractables were found in the IPA/water extract (Fig 5), which was significantly more than was seen for the other four solvents. HRAM data were processed using Thermo Scientific Compound Discoverer™ small-molecule structure analysis software to identify the compounds. This software 'predicts' structures and performs online library searches against the Royal Society of Chemistry's ChemSpider (RSC) and HighChem's mzCloud databases, as well as against a local extractables and leachables compound database. As before, the major peak observed from all of the extracts was that of DEHP.

The IPA/water extract was seen to contain higher concentrations of hydrophobic compounds. Two extractables in this extract had the same molecule weight and chemical composition but different elution retention times, indicating that they were structural isomers of each other. However, MS/MS analysis indicated a different fragmentation pattern and therefore running both full scan and MS/MS analysis will be necessary in order to determine the true situation.

Figure 5: Extractables in IPA/Water Extract



THE IMPORTANCE OF COMPREHENSIVE ANALYSIS

This study has shown how important it is to carry out careful, comprehensive analyzes that demonstrate how the plastic bioprocess bags used in biopharmaceutical production can contaminate products when in contact with liquids that can extract compounds from them. The risks that extractables may pose to patients can only be assessed and quantified by understanding what compounds might be extracted from bioprocess bags and how much of each of these compounds is likely to be extracted under various conditions.

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