Volume 23 Number 4

BioPharm INTERNATIONAL

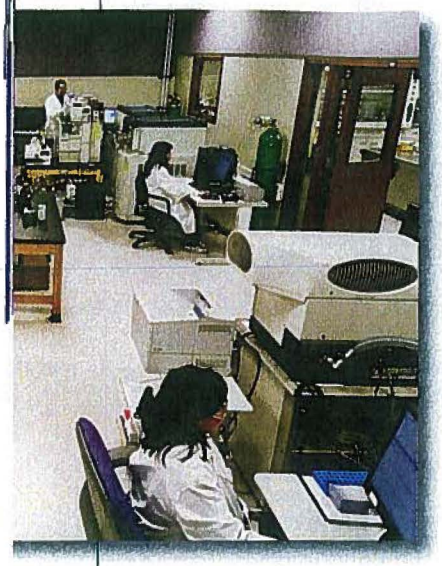
April 2010

The Science & Business of Biopharmaceuticals

www.biopharminternational.com

AN ADVANSTAR PUBLICATION

FINAL WORD: Biotech Needs Policies that Encourage Innovation 50



PPD, Inc.

A Study of Leachables for Biopharmaceutical Formulations Stored in Rubber-Stoppered Glass Vials

Xiaochun Yu, Don DeCou, Derek Wood, Steven Zdravkovic, Heather Schmidt, Laurie Stockmeier, Robert Piccoli, Daniel Rude, Xiaoya Ding

ABSTRACT

This article describes a systematic approach to evaluating the leachables profile from various biopharmaceutical formulations stored in rubber-stoppered glass vials. We evaluate leachables from rubber stoppers in 11 different formulations containing typical biopharmaceutical excipients to assess how differences in various formulation excipients affect leachables. The information obtained from this type of study is critical to support the Quality by Design paradigm of incorporating product quality into the product design process and using risk-based approaches to managing quality.

Xiaochun Yu, PhD, is a senior research scientist, Don DeCou, PhD, is a senior research scientist, Derek Wood is a laboratory manager, Steven Zdravkovic is a senior scientist, Heather Schmidt is an associate research scientist, Laurie Stockmeier is a scientist, Robert Piccoli, PhD, is an associate research scientist, Daniel Rude is a research scientist, and Xiaoya Ding, PhD, is the director of scientific and technical affairs, all at the cGMP laboratory, PPD, Inc., Wilmington, NC, 910.558.7585, xiaoya.ding@ppdi.com.

Biopharmaceutical packaging materials (container/closure systems) must be evaluated for compatibility with the drug formulation in the early stages of development to ensure the product is safe for use throughout its shelf life. This requirement also applies to the development of biopharmaceutical manufacturing processes including single-use components used in manufacturing equipment and in-process production conditions.[1] Although compendial tests provide some preliminary extractables information about the container/closure systems, they do not identify or quantitate individual extractables. Therefore, they cannot correlate extractables from the container/closure systems to the leachables in the drug products. This article describes a systematic approach to determining extractables and leachables based on the concepts proposed by the Product Quality Research Institute's Leachables and Extractables Working Group for the development of orally inhaled and nasal drug products in concert with relevant regulatory guidelines.[2–4] The study design focuses on identifying and elucidating the correlation between leachables caused by biopharmaceutical formulations stored in rubber-stoppered glass vials and its potential application in supporting Quality by Design (QbD) in the biopharmaceutical development process.

Table 1. Aqueous formulations used for leachables evaluations

Formulation number	Formulation	Buffer (20 mM)	Bulking agent	Stabilizer	Tonicity modifier	Chelating agent	Surfactant	Co-solvent
1	Phosphate buffer	pH 6.8 phosphate						
2	Buffer with co-solvent	pH 6.8 phosphate						2% glycerol
3	pH variations	pH 5.0 citrate	7% sucrose	Sucrose	150 mM NaCl			
4		pH 6.8 phosphate	7% sucrose	Sucrose	150 mM NaCl			
5		pH 8.2 phosphate	7% sucrose	Sucrose	150 mM NaCl			
6	Chelating agent	pH 6.8 phosphate	7% sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA disodium		
7		pH 6.8 phosphate	7% sucrose	Sucrose	150 mM NaCl	0.5 mM EDTA disodium		
8	Surfactant	pH 6.8 phosphate	7% sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA disodium	0.1% Tween 80	
9		pH 6.8 phosphate	7% sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA disodium	0.5% Tween 80	
10	Bulking agent	pH 6.8 phosphate	7% mannitol		150 mM NaCl			
11		pH 6.8 phosphate	7% trehalose	Trehalose	150 mM NaCl			

EDTA: ethylenediaminetetraacetic acid

Glass vials and rubber stoppers are widely used as container/closure systems for biopharmaceutical products and other drug formulations. Organic compounds in the stoppers, such as oligomers, antioxidants, and curing agents, can leach out into drug formulations and affect drug safety and efficacy. In addition, typical biopharmaceutical drug product formulation ingredients, such as co-solvents, surfactants, chelating agents, bulking agents, and pH modifiers, can alter the physicochemical properties of the drug formulation itself and also may have an impact on the leaching of organic compounds from rubber stoppers. This paper evaluates leachables from rubber stoppers in 11 different formulations containing typical biopharmaceutical excipients, and addresses how differences in various formulation excipients affect leachables.

In this study, typical biopharmaceutical formulation components were chosen to create a variety of test formulations. The extractables profiles of chlorobutyl rubber stoppers were determined. The major extractables were then chosen as targets for a leachable assessment in which the test formulations were stored at 40 °C and 75% relative humidity (RH) for one month. The following formulation variations were evaluated: pH (5.0, 6.8, 8.2), chelating agent (0.1 to 0.5 mM EDTA), surfactant concentration (0.1–0.5% Tween 80), bulking agent (sucrose, mannitol, or trehalose), and the presence of a co-solvent (2% glycerol).

STUDY DESIGN

Eleven aqueous placebo formulations were evaluated in this study (Table 1). The various test formulations included a simple phosphate buffer at pH 6.8, a buffer with a glycerol co-solvent, three formulations in which the pH was varied from slightly acidic to slightly alkaline, two formulations with different amounts of ethylenediaminetetraacetic acid (EDTA) included as a chelating agent, two formulations with different amounts of Tween 80 included as a surfactant, and two formulations with

either mannitol or trehalose added as bulking agents. Commercially available glass vials and rubber stoppers were used in the study.

Separate glass vials were filled with one of the 11 formulations listed in Table 1 and crimp-sealed with rubber stoppers. The stoppered vials containing various formulations were inverted and then stored at 40 °C and 75% RH. After one month, the formulations were tested for volatile, semivolatile, and nonvolatile leachables using headspace gas chromatography–mass spectrometry (GC–MS), direct injection GC–MS, and gas chromatography–ultra violet mass spectrometry LC–UV–MS, respectively. The portions of each sample to be analyzed were transferred to headspace GC vials and high performance liquid chromatography (HPLC) vials without further treatment for headspace GC–MS and LC–UV–MS analysis, respectively. For direct injection GC–MS analysis, the samples were back extracted into methylene chloride, which is a more suitable solvent for this analysis. Control samples (portions of each formulation that were stored in glass volumetric flasks at 5 °C for the same time duration as the samples) also were analyzed.

To correlate the leachables with the extractables from the stoppers, the stoppers were extracted with water and isopropanol and analyzed with the same headspace GC–MS, direct injection GC–MS, and LC–UV–MS conditions. For headspace GC–MS analysis, the stoppers were extracted in headspace GC vials at 90 °C for 24 h, and the vials were directly used for volatile extractables analysis, without opening the vial cap. All volatile extractables, including water insoluble extractables, were detectable using this approach. For direct injection GC–MS and LC–MS analyses, the stoppers were extracted by refluxing with water and isopropanol for 16 h. Tables 2–4 show the chromatographic conditions used for each analysis.

Table 2. Headspace GC–MS chromatographic conditions

Column	DB-VRX 30 m × 0.25 mm, 1.4 µm film thickness or equivalent
Oven program	35 °C (15 min), 10 °C/min, 220 °C (10 min)
Injector port temp	220 °C
Flow (He)	1 mL/minute (constant flow)
Split ratio	5:1 (split flow column flow)
Run time	43.5 min
Mass range	35–350 amu
Ionization	EI
Oven temperature	75 °C

GC–MS: gas chromatography–mass spectrometry

Table 3. GC–MS chromatographic conditions

Instrument	Agilent 5973 GC-MSD
GC column	HP-5MS, 30 m, 0.25 mm id, 0.25 µm film thickness or equivalent
Flow	1.0 mL/min helium
Temperature program	40 °C (2 min), 15 °C/min, 320 °C (20 min)
Mode	Splitless
Purge time	0.5 min
Inlet temperature	280 °C
Injection volume	1.0 µL
Mass range	35–650 amu

GC–MS: gas chromatography–mass spectrometry

Table 4. LC–MS chromatographic conditions

UPLC system	Waters Acquity
MS detector	Waters Acquity TQD
Mobile phase A	Water
Mobile phase B	Acetonitrile
Column	Waters Acquity UPLC BEH C18, 2.1 × 50 mm, 1.7 µm
Flow rate	0.8 mL/min
Column temperature	60 °C
UV wavelength	280 nm
Injection volume	5 µL
Gradient	Water and acetonitrile gradient
Ionization mode	APCI (–) and APCI (+)
Mass range	150–1,200 amu

LC–MS: liquid chromatography–mass spectrometry

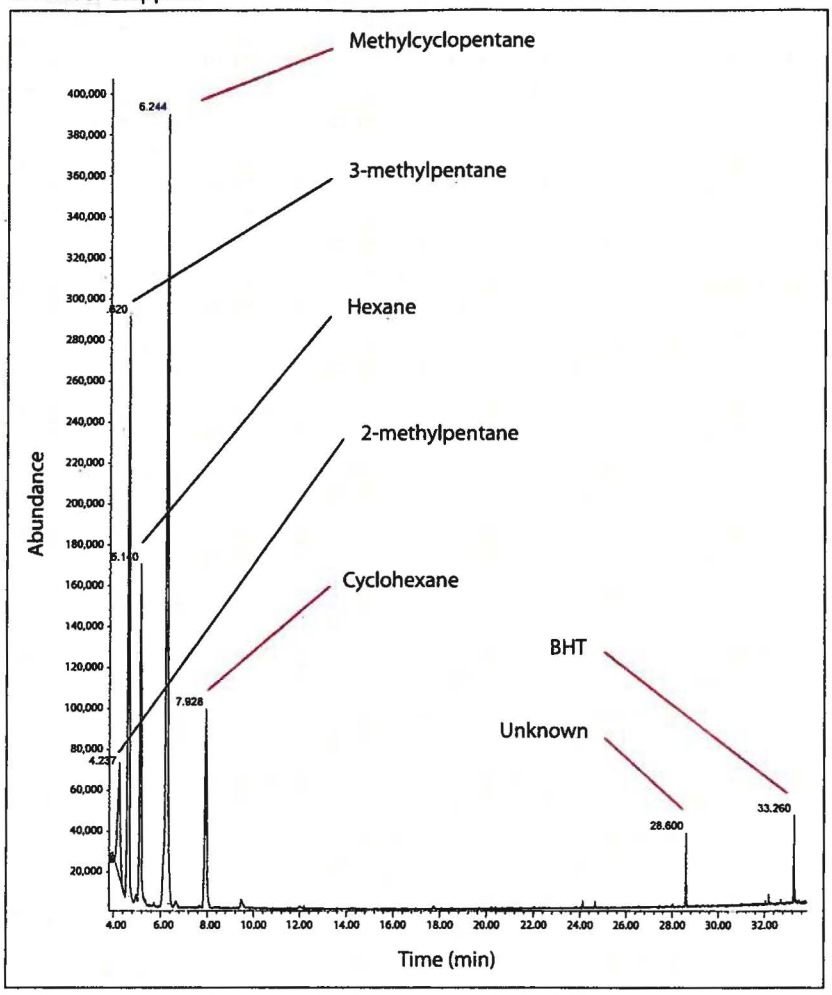
RESULTS AND DISCUSSION

Headspace GC–MS Analysis

Extractables Evaluation

The water extracts of stoppers yielded seven extractables peaks (Figure 1). Six of the seven peaks were identified as 2-methylpentane (4.24 min), 3-methylpentane (4.62 min), hexane (5.14 min), methylcyclopentane (6.24 min), cyclohexane (7.93 min), and butylated hydroxytoluene (BHT, 33.26 min). The peak at 28.60 min was not identified; the GC–MS library search and manual spectral interpretation did not produce a good match or a tentative identification. The amounts for each peak are summarized in Table 5. The peak at 28.60 min was quantitated using cyclo-

Figure 1. Headspace GC–MS chromatogram of water extracts of rubber stoppers



GC–MS: gas chromatography–mass spectrometry; BHT: butylated hydroxytoluene

hexane as the surrogate standard. Because the relative response factor of the unknown peak against cyclohexane is not determined, the amount reported for this peak is only considered a semi-quantitative estimate.

Leachables Evaluation

Six leachables peaks were observed in the headspace GC–MS analysis from the formulations: 2-methylpentane, 3-methylpentane, hexane, methylcyclopentane, cyclohexane, and BHT. These leachables peaks correlate to stopper extractables. The unknown extractables at 28.60 min were not observed as leachables. This may be because of insolubility of the compound in the aqueous media. The amount of leachables in different formulations is summarized in Table 5. The effect of various formulation ingredients on the leachables profile is discussed in detail below.

Phosphate Buffer with or without Glycerol Co-Solvent

Five leachables peaks were observed in the pH 6.8 phosphate buffer: 3 methylpentane, hexane, methylcyclopentane, cyclohexane, and BHT. All of the peaks were very small. Methylcyclopentane

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.