

PURPOSE

To evaluate four aspects of the BioPhorum Operations Groups (BPOG) proposed study design:

- 1) Length of extraction time
- 2) Use of a dynamic versus a static extraction
- 3) Timing of the initiation of the set down
- 4) Impact of freezing samples before analysis

METHOD

The intent of the BPOG protocol is to standardize extractables testing for single-use systems. In this study the BPOG protocol, in conjunction with Eurofins Lancaster Laboratories screening analyses, was applied to a 500 mL polyethylene (PE) bag using 2.5M acid and base solutions. Several different extraction designs were performed to evaluate different attributes of the BPOG protocol including (1) the length of extraction time, (2) the need for agitation during extraction, (3) the use of staggered extraction times, and (4) the impact of freezing.

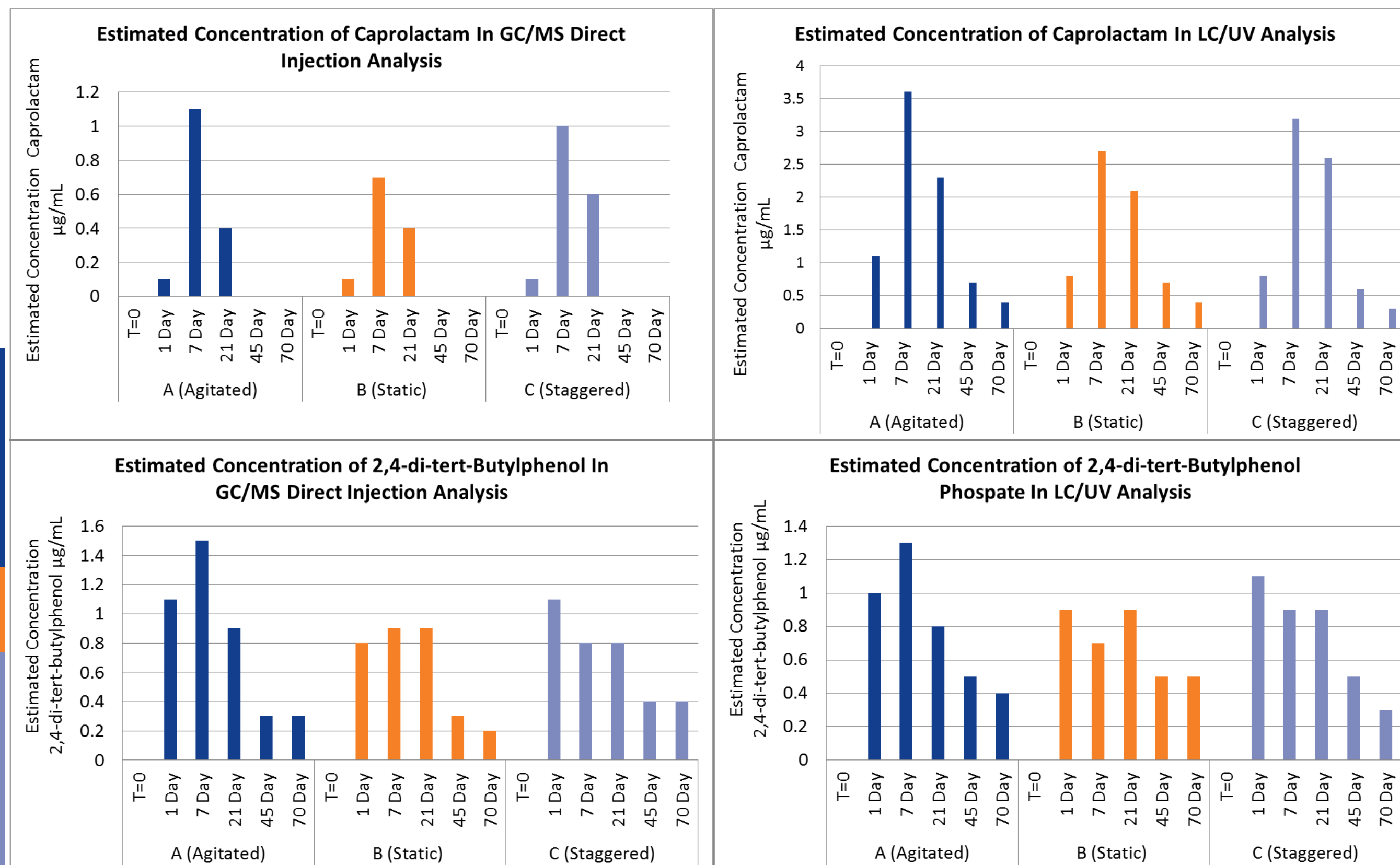
Extraction A mimicked the proposed BPOG conditions. PE bags were filled with 100 mL of either 2.5 M phosphoric acid or 2.5 M sodium hydroxide. Air was expelled from the bag to maximize exposure of the bag film. The bags were then placed in an incubating shaker at 40°C, and agitated at 50 rpm. The resulting extracts were analyzed at six different time points: <30 minutes, 24 hours, 7 days, 21 days, 45 days and 70 days. In addition, the extracts from all time points were frozen in Teflon containers and reanalyzed at the 21-day and 70-day time point.

Extraction B evaluated a second set of PE bags that were extracted without agitation. All other aspects of Extraction B were identical to Extraction A.

Extraction C evaluated a third set of PE bags that were extracted using a staggered setup. In the staggered setup, the staging of the bags was designed so that all bags completed their necessary incubation at the same time; therefore, every time point could be analyzed at the same time. This staggered design was in contrast to Extraction A, in which the incubations for each time point began on the same day but ended at different times. All other aspects of Extraction C were identical to Extraction A.

RESULTS

The resulting extracts were analyzed by two analytical techniques. Liquid chromatography with time-of-flight mass spectrometric detection (LC/MS-TOF) was performed utilizing an Agilent 6200 series LC/MS-Time of Flight (TOF) equipped with a multimode source (electrospray and atmospheric pressure chemical ionization) and used positive ionization (MMP) and negative ionization (MMN). In addition, UV spectra were acquired from 190-500 nm using a photodiode array detector. Gas chromatography with mass spectrometric detection (GC/MS) was performed using direct injection sample introduction technique. GC/MS analysis was performed on an Agilent 7890B Gas Chromatograph / Agilent 5977A Mass Spectrometer. The GC/MS direct inject analysis utilized a mass spectrometer scan range of 30-550 amu.



CONCLUSION

The BPOG protocol to standardize extractables testing provides a solid structure for suppliers and biopharmaceutical companies to characterize single use systems. However, the study design presents logistical issues for laboratories performing these studies, including the capacity required to perform agitation on all samples, the capacity to handle the number of extracts generated, and the resources required to perform the study. The results of this study support the following protocol modifications or study designs to help offset cost and logistical concerns:

- No additional extractables were detected beyond the 21-day time point. The necessity to include 45 and 70 day time points in the study design should be evaluated.
- The results of this study supported the use of a staggered set down, which could be a viable option to offset costs by consolidating the analysis of all samples at the end of the study. *Note that in order to support a staggered extraction, planning is required to have the materials sterilized (gamma-irradiated or autoclaved) within the protocol-specified timeframes.
- Freezing extracts at each time point and analyzing at the final time point is another viable option supported by the results of this study.
- Finally, the requirement to agitate, which can be a logistical challenge, is not supported by this study.