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# The Practical Application of BPOG E&L Protocols to Viral Clearance Filters

Jessica Shea, Paul Killian, Ph.D, Thomas Stone, Ph.D

## Abstract

Regulatory guidance advocates virus control at various stages of the drug manufacturing process and directs that you test the capacity of the process to remove or inactivate virus. Patient safety concerns require you to determine what impurities may be added by the virus control steps you implement. While the application of a standardized approach to identifying and quantifying the extractables from these steps has benefits when making comparisons, choices have to be made when developing the protocol that take into account the characteristics of the clearance device and use conditions. This poster will illustrate the practical implementation of standardized extractables method on an industry leading viral clearance technology by explaining the rationale for the selection of extraction solvents; extraction conditions and sampling points. Data generated during the study is presented as well as lessons learned in implementing the new protocol.

## Study Objectives

- Evaluate the standardized extractables testing protocol that was introduced by the Biophorum Operations Group (BPOG).
- Refresh extractables data for an existing filtration device.
- Evaluate the feasibility of using an external laboratory resources to generate data.



## Study Design

**Viresolve® Pro Modus 1.2 Device**  
Catalog # VPMD102NB1  
Contains 0.07 m2 of Filtration Area  
Contains ~125 mL of "hold-up" volume

**Materials of Construction**  
Polyethersulfone (PES) membrane  
Polyvinylidene fluoride (PVDF) housing  
Silicone O-rings



## Procedures

- Per the User Guide, the devices were pre-wet with 3.5 L of WFI (350 mL/min for 10 minutes).
- The devices were filled with 125 mL of one of the four model solvents and the ports sealed with end caps.
- The device was then stored at either 25°C or at 40°C.
- At the end of the storage time, the device was recirculated with an additional 125 mL of fresh solvent for 5 minutes to completely homogenize the extraction solution. (Total extraction volume 250 mL).
- Three (3) replicates, each from a different lot, were tested per solvent per time point. (Total of 36 devices)

## Extraction Solvents

- ✓ WFI
- ✓ 0.5 N Sodium Hydroxide
- ✓ 0.1 M Phosphoric Acid
- ✓ 5 M Sodium Chloride
- ☐ 50% Ethanol in Water ← Evaluated but not tested
- ☐ 1% Polysorbate 80 ← Incompatible with Filter

## Time Points and Temperature

- 30 Minutes @ 25°C
- 1 Day @ 40°C
- 7 Days @ 40°C

## Analysis

- |                            |                                 |
|----------------------------|---------------------------------|
| <b>BPOG Required</b>       | <b>EMD Millipore Additional</b> |
| ✓ GC/MS – Headspace        | ✓ pH                            |
| ✓ GC/MS – Direct Injection | ✓ TOC                           |
| ✓ HPLC-DAD                 | ✓ Ion Chromatography            |
| ✓ HPLC-MS (ESI +/- modes)  | ✓ FTIR                          |
| ✓ HPLC-MS (APCI +/- modes) |                                 |
| ✓ ICP-MS                   |                                 |

## Acknowledgements:

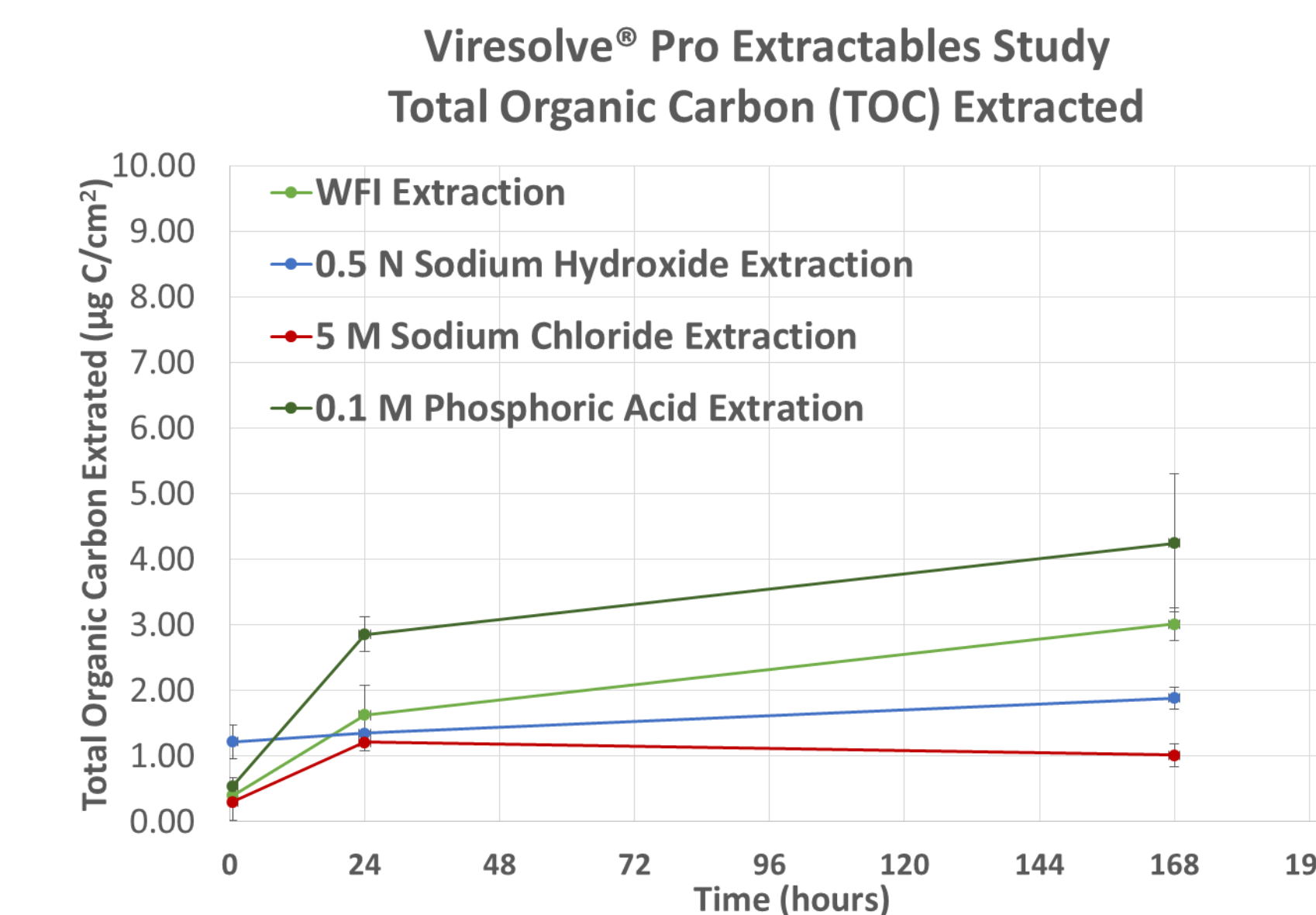
Special thanks to Chemic Laboratories

## Results

### Total Organic Carbon (TOC) Results

TOC results for each model solvent stream with timepoints of <30 minutes, 24 hour and 7 day timepoints. Results are the average plus standard deviation

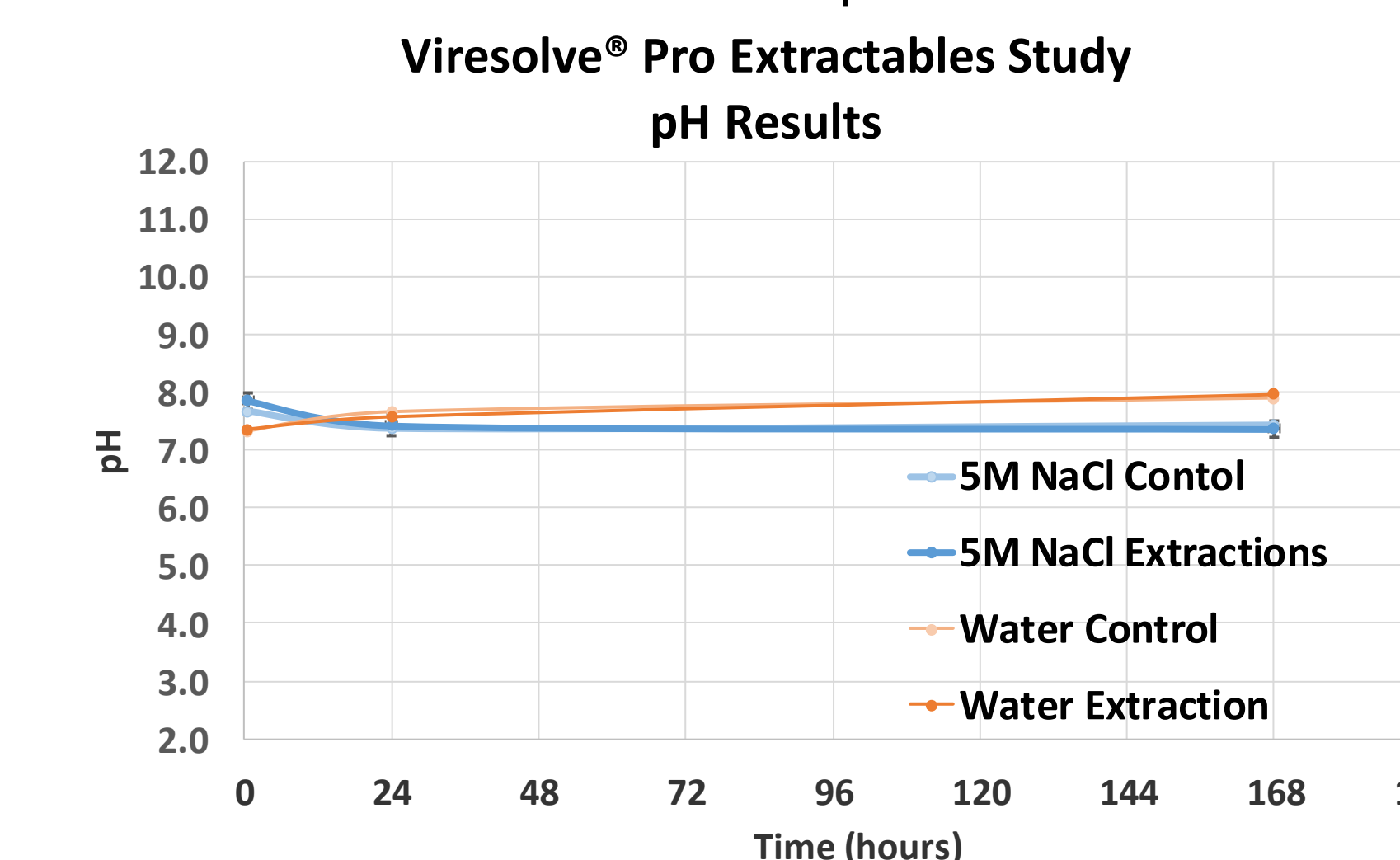
- Ranging from highest to lowest concentration  
0.1M H<sub>3</sub>PO<sub>4</sub> > WFI > 0.5N NaOH > 5M NaCl
- Values increased with time.
- Control not subtracted. The controls values were inconsistent due to drift and sample being run by timepoint. In some case the controls were above the samples.



### pH Results

Average pH readings of samples and control results for each model solvent stream with timepoints of <30 minutes, 24 hour and 7 day timepoints.

- There were no differences observed between the controls and the extracted samples



### HPLC-DAD-MS

- No peaks were identified in the following model solvent streams:
  - Water
  - 5M Sodium Chloride
  - 0.1M Phosphoric Acid
  - NaCl interference on Internal Standard (IS). Therefore, the laboratory had to find an acceptable alternative IS standard.
- Two small peaks were observed in the 0.5N Sodium Hydroxide Extractions in HPLC-DAD. However, prior to analysis on HPLC-MS the laboratory performed a multi-step solvent exchange. These two peaks were not present in the HPLC-DAD-MS analysis, and therefore could not be identified.

## Metals

The metals found were separated into three categories:

- Random = the metal was not consistently present in the extracts.
- Noisy baseline= These results were just above the instrument detection limit and also present in several of the control sample.
- Cross Contamination = These metals were observed at significant concentration in the control samples

### Water Extraction

- Random detection of Aluminum, Calcium, and Potassium
- Noisy Baseline for Vanadium

### 0.1M H<sub>3</sub>PO<sub>4</sub> Extraction

- Random detection of Barium, Chromium, Copper, Manganese, and Tin
- Cross Contamination (present in the controls) Aluminum, Antimony, Titanium, and Zinc
- Noisy Baseline for Vanadium and Selenium

### Detection of Sodium Chloride

Sodium was detected in all samples and controls, suspected contamination from the 5M Sodium Chloride Extraction.

#### Sodium results in the Water Extraction

Timepoint	QL	Control	Lot 1	Lot 2	Lot 3
0	200	380	740	810	440
1	200	360	560	1000	630
7	200	490	720	560	420

#### Sodium results in the 0.1M H<sub>3</sub>PO<sub>4</sub> Extraction

Timepoint	QL	Control	Lot 1	Lot 2	Lot 3
0	200	670	12000	5800	1100
1	200	440	2900	3900	4600
7	200	860	1600	1200	1500

All results in µg/L

### Other Analysis

No Extractables Identified by:

- Ion Chromatography (IC)
- Headspace GC/MS
- Direct Injection GC/MS
- Fourier transform infrared spectroscopy (FTIR)

## Conclusion

Due to materials of construction, lack of sterilization, and a pre-flush study very few extractables were expected. And very few extractables were found.

Optimization of the BPOG methods is required to achieve:

- A more robust TOC analysis;
- An improved metals interpretation;
- And eliminate the need for solvent exchange for HPLC-MS.

An external laboratory study will take ~ 4 months and requires preparation and close coordination.

## Lessons Learned

- External studies require thorough preparation and coordination. Additional preparation around nomenclature and sequence order would have been useful.
- A pre-use flush will greatly affect results.
  - "If an item is pre-treated prior to actual use, the item should be pre-treated in the same way before being used in extractables testing."
- Learning Curve with 5M Sodium Chloride
  - TOC baseline stability
  - Cross contamination in ICP analysis
  - Interference with HPLC internal standard