

Characterization of Extractables from Sartoflow® Single-Use Tangential Flow Filtration (SUTFF) Flow Kits

Extractables Report Summary –
(20% Dimethylacetamide | 80% Water Solution)



Technical Note

Confidential Technical Report
for Sartoflow® 150, 1000,
and 4500

1. Summary

An extractables study was performed on the Sartoflow® single-use tangential flow filtration (SUTFF) flow kits using 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution as the extraction fluid. The data was generated to provide an evaluation of the types of extractables that could be extracted from the SUTFF flow kits. This would enable an assessment of potential leachables that may be present when the flow kits are in contact with an aqueous drug substance during processing. The test systems were designed to contain all the single-use components which are part of the feed, permeate and retentate flow kits of a ½ inch standard SUTFF system apart from the SUTFF plates and are therefore representative of the entire fluid pathway of Sartoflow® SUTFF system.

Prior to extraction, the SUTFF flow kits were gamma irradiated at 50 ± 5 kGy. The SUTFF flow kits were extracted for an initial 4 hour period with 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution at 27.5 ± 2.5 °C. After the end of the first exposure, the test systems were drained and to the pre-extracted systems was added a fresh amount of 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution at 27.5 ± 2.5 °C for a further 4 hours.

The extraction fluids from the first and second exposure periods were subjected to detailed analyses including Non-Volatile Residue (NVR), Fourier Transform Infrared Spectroscopy (FTIR), Direct Injection Gas Chromatography | Mass Spectrometry (GC | MS), Headspace GC | MS and Inductively Coupled Plasma | Mass Spectrometry.

Non Volatile residue (NVR) analysis showed no more than 3.2 mg was obtained from the first extraction. Non Volatile residue (NVR) analysis showed no more than 1 mg was obtained from the second extraction.

Fourier Transform Infrared (FTIR) analysis was performed on the NVR. The results of this determined that there was insufficient material extracted from the systems to be identified by FTIR.

Headspace gas chromatography | mass spectrometry (HS | GC | MS) analysis determined that trimethylsilanol was present in the samples at no more than 1.1 µg/mL or 3.3 mg in the first extraction. In the second extraction, trimethylsilanol was detected at no more than 0.6 µg/mL or 1.9 mg.

Direct Injection gas chromatography | mass spectrometry analysis (GC | MS) revealed that there were no semi-volatile compounds detected from the test systems.

Further analysis of the flush extracts by Liquid Chromatography | Ultraviolet | Mass Spectrometry (LC | UV | MS) determined that there were no non-volatile compounds present in the samples.

Elemental analysis of the sample extracts determined the presence of the following metals at a maximum concentration: Aluminum 21 ng/mL or 0.063 mg, Barium 0.4 ng/mL or 0.0012 mg, Calcium 160 ng/mL or 0.48 mg, Sodium 100 ng/mL or 0.3 mg, Magnesium 250 ng/mL or 0.75 mg, Titanium 1.1 ng/mL or 0.0033 mg, Zinc 120 ng/mL or 0.36 mg, and Lead 33 ng/mL or 0.099 mg.

A summary of the characterization of the extractables from the SUTFF flow kits after extraction are presented in Table 1.

Table 1

Characterization of the Extractables from the SUTFF Flow Kits Using 20% Dimethylacetamide (DMAC) | 80% Water as an Extraction Fluid.

Analyses	Analytical Methods	Extractables After 1st Extraction	Extractables After 2nd Extraction
Non Volatile compounds	NVR	3.2 mg	1 mg
Oligomers	FTIR	Insufficient residues isolated for characterization	Insufficient residues isolated for characterisation
Volatile compounds	Headspace GC MS	Trimethyl silanol 1.11 ppm or 3.33 mg	Trimethyl silanol 0.62 ppm or 1.86 mg
Semi-volatile compounds	Direct Injection GC MS	< Limit of Detection	< Limit of Detection
Semi-volatile and Non-volatile compounds	LC UV MS	< Limit of Detection	< Limit of Detection
Metals	ICP MS	Al 8 ppb or 0.024 mg, Ba 0.4 ppb or 0.0012 mg, Ca 160 ppb or 0.48 mg, Na 100 ppb or 0.3 mg, Mg 21 ppb or 0.063 mg, Ti 1.1 ppb or 0.033 mg, Zn 120 ppb or 0.36 mg, Pb 33 ppb or 0.099 mg	Al 21 ppb or 0.063 mg, Ba 0.6 ppb or 0.0018 mg, Ca 110 ppb or 0.33 mg, Na <LOQ, Mg 24 ppb or 0.072 mg, Ti <LOQ, Zn 3 ppb or 0.009 mg, Pb 7 ppb or 0.021 mg

2. Introduction

Sartoflow® SUTFF flow kits form the fluid contact components of the SUTFF automated systems which are designed for biopharmaceutical applications such as concentration of biomolecules, desalting protein solutions, purification and recovery of monoclonal antibodies (mAb) or recombinant proteins. This study was conducted to provide final users of Sartoflow® SUTFF systems with information regarding the extractables coming from the polymeric flow kits using 20% DMAC | 80% ultrapure water solutions as the extracting fluid.

The test systems used for the extraction studies contains all the single-use components which form part of the feed, retentate and permeate of the SUTFF system flow kits apart from the SUTFF distribution plates. These components are all included in the standard flow kits that are supplied with the Sartoflow® SUTFF system.

A combination of several analytical methods used to evaluate extractables from the SUTFF flow kits are listed in Table 2.

Table 2
Analytical Methods Used for the Assessment of Extractables

Analytical Method	Target Compounds Property
Non Volatile Residue (NVR)	Total mass of extractables after test solvent evaporation
Fourier Transform Infrared (FTIR) Spectroscopy	Oligomers of the materials of construction and functional group identification of the materials in the NVR
Gas Chromatography Mass Spectrometry (GC MS) – Headspace	Volatile organic compounds that may come from monomers and oligomers, residual solvents from various production steps, additives, residues from polymer treatment and degradation products
Gas Chromatography Mass Spectrometry (GC MS) – Direct Injection	Semi-volatile compounds including lubricants, plasticizers, antioxidants and residual solvents with higher boiling points
Gas Chromatography Mass Spectrometry (GC MS) – Derivatization	Organic acids including fatty acids
Liquid Chromatography Ultraviolet Mass Spectrometry (LC UV MS)	Non-volatile organic compounds (and some semi-volatile organic compounds) including hindered phenolic antioxidants and plastic additives such as plasticizers, anti-slip agents, fatty acids, and other plastic processing aids
Inductively Coupled Plasma Mass Spectrometry (ICP MS)	Metals that may come from catalysts used in the polymerization process and plastic additives

3. Materials, Reagents and Equipment

The materials and equipment used in this study are listed in Tables 3a-b.

Table 3a
Equipment

Equipment	Description
Extraction equipment	Graduate glass cylinders (2-Liter), PTFE (Polytetrafluoroethylene)-lined diaphragm pump, rotary flash-evaporator, vacuum pump, oil and water bath, glass vessels, desiccators, drying oven
FTIR (Fourier Transform Infrared Spectroscopy)	Shimadzu IR Prestige 21 - serial number: A21004602202
Gas Chromatography Mass Spectrometry (GC MS)	Aligent 5973 5975 5977 Mass spectrometer coupled to Aligent 6890 7890 gas chromatograph
Liquid Chromatography Ultraviolet Mass Spectrometry (LC UV MS)	Waters Xevo Mass spectrometer Waters Acquity UPLC system with DA detector
Inductively Coupled Plasma Mass Spectrometry (ICP MS)	Agilent 7500 cs

Table 3b
Solvents and Reagents

Solvent Reagent	Manufacturer (Lot Number)
Potassium Acid Phthalate (KHP)	Merck (L N A803574731)
2-Fluorobiphenyl	Sigma (L N MKBF8275V)
Butylated Hydroxy Toluene (BHT)	Sigma (L N S00632-121)
Octadecane	Sigma (L N BCBC1527V)
Bis (2-ethylhexyl)phthalate	Sigma (L N SZBV167XU)
2,4-di- <i>tert</i> -butyl phenol	Sigma (L N S43419-259)
Irganox 245	Ciba (L N 15262FC4)
Oleamide	Sigma (L N SLBB6213V)
Irganox 1035	AccuStandard (L N 13058)
Irganox 259	AccuStandard (L N 5687)
Irganox 1010	Ciba (L N 02157FC7)
Irganox 1076	AccuStandard (L N 07003JH)
Irgafos 168	AccuStandard (L N 13802PO)
Hexadecanoic Acid	Sigma (L N 90719)
Ocatadecanoic Acid	Sigma (L N 11k5319)
Dimethylacetamide	VWR Hipersolv (L N 14Z1058)

4. Test Methods

Table 4

Test Parameters

Test Solvent	20% Dimethylacetamide (DMAC) 80% ultrapure water solution
Test Temperature	27.5 °C ± 2.5 °C
Test System P N	739-54C, 739-54S, 739-54R
Number of Systems	2
Pre-sterilization Method	50 ± 5 kGY
Extraction Method	Recirculation
Test Solvent Volume	3 L
Surface Area (m²)	0.056
Flow Rate	2.7 L/min

Preparation

Equipment cleanliness: All equipment was cleaned and rinsed with DI water prior to use.

4.1 Extraction Method

Prior to extraction, the test systems were gamma irradiated to 50 ± 5 kGY. This level of gamma irradiation was selected for the test systems as this is the worst case gamma irradiation dose that production systems will receive. The production systems are typically gamma irradiated at a minimum of 25 kGy and no more than 50 kGy.

The test systems were designed to contain all the single-use components which are part of the feed, permeate and retentate of the SUTFF flow kits other than the SUTFF distribution plates. The test systems parts, 739-54C, 739-54S, 739-54R were connected together and then the single-use Quattroflow* pump head which is part of 739-54S was installed onto a Quattroflow pump hardware. Each end of the system was placed in a 5 L clean glass beaker which contained 3 L of 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution, thereby forming a continuous recirculation loop. The test systems were extracted for 4 hours at 27.5 °C ± 2.5 °C. After this first extraction period the test systems were emptied. The pre-extracted systems were then extracted using another 3 L of 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution for a further 4 hours.

A negative control sample of 20% Dimethylacetamide (DMAC) | 80% ultrapure water solution was handled in parallel but without any contact with the test systems and analysed appropriately. A known amount of potassium hydrogen phthalate (KHP) was dissolved in ultrapure water solution and analysed by NVR and FTIR as a positive control.

4.2 Analysis of the Extracts

4.2.1 Non-volatile Residue (NVR)

An aliquot of the negative control and the 1st and 2nd extracts were evaporated separately in a 2 L round-bottom flask until the volume was reduced to less than 10 mL. Each concentrated sample was then transferred into a clean pre-weighed crucible, including solvent rinses and dried to a constant weight in a dessiccator using a drying oven set at 60 °C.

4.2.2 Fourier Transform Infrared (FTIR) Spectroscopy

The NVR from the extracts and the negative control were analyzed by FTIR (Fourier Transform Infrared Spectroscopy). Spectral libraries supplied with the instrument were used to obtain reference spectra for comparison, and spectral matches (where applicable).

4.2.3 Gas Chromatography | Mass Spectrometry – Headspace

The test system extracts and the negative control were analyzed for volatile organic compounds in duplicate per the parameters listed in Table 5. System suitability (detector sensitivity and injection precision) was verified using a standard solution of benzaldehyde prior to sample analysis.

Table 5

GC | MS Headspace Test Parameters

GC Column	BPX volatiles, 60 m × 0.25 mm, 1.40 µm
Mass Range	40 to 700 amu (atomic mass unit)
Injection Volume	1 mL
Injection Port Temperature	250 °C
Column Temperature Profile	(a) 50 °C for 0.5 minutes (isothermal); (b) heating at 10 °C/min to 240 °C held for 10 mins

4.2.4 Gas Chromatography | Mass Spectrometry – Direct Injection

The test system extracts and the negative control were analyzed for semi-volatile organic compounds in duplicate per the parameters listed in Table 6. System suitability (detector sensitivity and injection precision) was verified using a standard solution of 2,4-di-*tert*-butyl phenol prior to sample analysis.

*Quattroflow is a trademark of Almatec Maschinenbau GmbH

Table 6

GC | MS Direct Injection Test Parameters

GC Column	Agilent DB-Wax, 30 m × 0.25 mm, 0.25 µm
Mass Range	40 to 700 amu (atomic mass unit)
Injection Volume	1 µL
Injection Port Temperature	280 °C
Column Temperature Profile	(a) 60 °C for 0.5 minutes (b) heating at 15 °C/min to 250 °C held for 10 minutes.

4.2.5 Liquid Chromatography | Ultraviolet | Mass Spectrometry (LC | UV | MS)

The extracts and the negative control were analyzed for non-volatile organic compounds (and some semi-volatile organic compounds) in duplicate per the parameters listed in Table 7. System suitability (detector sensitivity and injection precision) was verified using a standard solution of common plastic additives - Oleamide, 2,4-di-*tert*-butyl phenol, Irganox 259, Irganox 1010, Irganox 1076, Irgafos 168, Irganox 1035, Irganox 245 and BHT prior to sample analysis.

Table 7

LC | UV | MS Test Parameters

UHPLC Column	Kinetex, 5 µm, 2.1 × 50 mm	
Detection	Mass Spectrometry, Photodiode Array (PDA)	
Flow Rate	0.5 mL/min	
Column Temperature	60 °C	
Injection Volume	5 µL	
Mass Range	100 to 2000 amu (atomic mass unit)	
MS Ionization Modes	ES(+), ES(-) (ES- Electrospray)	
Mobile Phase	A: 5 mM Ammonium Formate B: Methanol	
Mobile Phase Gradient: Time	% A	% B
4	40	60
2	20	80
6	2	98
12	2	98
12.5	40	60

4.2.6 Inductively Coupled Plasma | Mass Spectrometry (ICP | MS)

The extracts and the negative controls were analyzed for Aluminum (Al), Arsenic (As), Barium (Ba), Beryllium (Be), Boron (B), Cadmium (Cd), Calcium (Ca), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Lead (Pb), Selenium (Se), Thallium (Tl), Titanium (Ti), Vanadium (V) and Zinc (Zn) by ICP | MS. Prior to analysis, the samples were acidified with concentrated hydrochloric acid.

5. Results

5.1 Non-Volatile Residue (NVR)

The extracts from test systems were concentrated down using a rotary evaporator and dried down in an oven until a constant weight for the non-volatile residue is achieved.

Total extractables as non volatile residue (NVR) from the negative control and the test systems extracts are shown in Table 8.

Table 8
NVR Results for Test Systems A & B

Sample	Weight of Non-Volatile Extracts (mg) Test System A	Weight of Non-Volatile Extracts (mg) Test System B
1st Extraction	3.0	3.2
2nd Extraction	< 1	< 1
Negative Control	< 1.0	< 1.0

As reported in Table 8 the NVR range was measured as 3.2 mg for the first extractions of the two test systems. By the second extraction, the NVR levels had reduced to below the detection limit as compared to the negative control.

5.2 Fourier Transform Infrared (FTIR) Spectroscopy

The infrared spectra of the extracts showed that residues from the samples were similar to the residues from the negative control and as such it was determined that there was insufficient material extracted from the systems to be identified by FTIR.

5.3 Gas Chromatography | Mass Spectrometry (GC | MS) – Direct Injection

No additional semi-volatile organic compounds compared to the blank were characterized by GC | MS direct injection.

5.4 Gas Chromatography | Mass Spectrometry (GC | MS) – Headspace

One volatile compound was detected by Headspace GC | MS. Trimethylsilanol was identified at levels of 1.1 µg/mL in the first extraction from Test System A and 1.1 µg/mL from the first extraction of Test System B. The levels of trimethylsilanol were reduced to 0.62 µg/mL in the second extraction of the test System A and 0.62 µg/mL in Test System B. These values are not corrected with the negative control, as levels in the negative control were found to be less than the Limit of Quantitation (LOQ). As a result the values listed in Table 9 are worst case.

As 3 L of 20% DMAC | 80% water was used for the system extraction, the maximum amount of trimethylsilanol identified during the 1st extraction was 3.3 mg and for the 2nd extraction was 1.86 mg.

Table 9
Summary of Compounds Detected by Headspace GC | MS

Amount in Sample (µg/mL)						
Retention Time (mins)	Compound Name	Control	A	A	B	B
			1st Extract	2nd Extract	1st Extract	2nd Extract
6.19	Trimethylsilanol	< LOQ	1.1	0.62	1.1	0.62

Testing performed in duplicate

5.5 Liquid Chromatography | Ultraviolet | Mass Spectrometry (LC | UV | MS)

The extracts were analyzed for non-volatile and semi-volatile organic compounds by LC | UV | MS. No different or extra peaks were detected by Electrospray either in positive or negative modes when compared to the negative control. This indicated that there were no non-volatile compounds present in the samples above 0.01 ppm (Limit of Detection (LOD) of the reference standard Irganox 1035).

5.6 Inductively Coupled Plasma | Mass Spectrometry (ICP | MS)

ICP | MS analysis was performed to determine the concentration of metal elements in the sample extracts.

* Note: If amount in the negative control > sample, the amount in sample was assumed to be < LOD after negative control subtraction.

The results are summarized in Tables 10 and 11.

Table 10

ICP | MS Results for Test System A (Results are in ng/mL)

Element	Mass	LOD	Negative Control	1st Extraction	2nd Extraction
Li	7	3	<LOD	<LOD	<LOD
B	11	10	<LOD	<LOD	<LOD
Na	23	100	200	300	300
Mg	24	3	23	42	46
Al	27	2	7	04	21
K	39	800	<LOD	<LOD	<LOD
Ca	40	80	360	420	420
Ti	48	4	2.5	3.2	3.0
Cr	52	20	<LOD	<LOD	<LOD
Mn	55	3	<LOD	<LOD	<LOD
Fe	56	20000	<LOD	<LOD	<LOD
Co	59	2	<LOD	<LOD	<LOD
Ni	60	20	<LOD	<LOD	<LOD
Cu	63	50	<LOD	<LOD	<LOD
Zn	66	5	<5	120	8
Sn	118	4	<LOD	<LOD	<LOD
Ba	137	1	0.2	0.4	0.6
Pb	207	3	<LOD	33	10

Table 11

ICP | MS Results for Test System B (Results are in ng/mL)

Element	Mass	LOD	Negative Control	1st Extraction	2nd Extraction
Li	7	3	<LOD	<LOD	<LOD
B	11	10	<LOD	<LOD	<LOD
Na	23	100	200	300	<LOD
Mg	24	3	23	24	23
Al	27	2	8	4	21
K	39	800	<LOD	<LOD	<LOD
Ca	40	80	360	<LOQ	<LOQ
Ti	48	4	2.5	<LOQ	<LOQ
Cr	52	20	<LOD	<LOD	<LOD
Mn	55	3	<LOD	<LOD	<LOD
Fe	56	20000	<LOD	<LOD	<LOD
Co	59	2	<LOD	<LOD	<LOD
Ni	60	20	<LOD	<LOD	<LOD
Cu	63	50	<LOD	<LOD	<LOD
Zn	66	5	<5	84	<LOD
Sn	118	4	<LOD	<LOD	<LOD
Ba	137	0.1	0.2	0.3	<LOD
Pb	207	0.3	<LOD	9	<LOD

6. Conclusions

Non volatile residue (NVR) analysis revealed that in the first extraction the maximum amount of non-volatile compounds present was 3.2 mg and in the second extraction, the amount was no more than 1 mg.

Direction injection gas chromatography | mass spectrometry (GC | MS) analysis showed that there were no semi volatile compounds present in the test system extracts.

Headspace gas chromatography | mass spectrometry (GC | MS) analysis identified trimethylsilanol present in the test systems. A maximum concentration of 1.1 µg/mL or 3.3 mg in the first extraction and 0.62 µg/mL or 1.86 mg in the second extraction was determined.

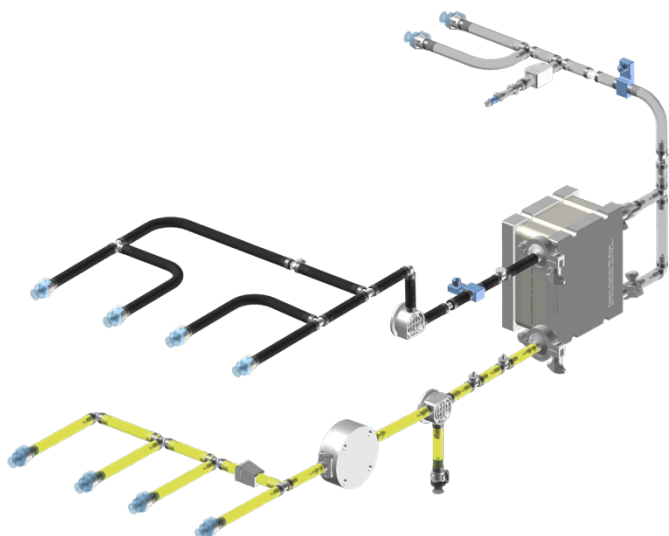
Liquid Chromatography | Ultraviolet | Mass Spectrometry (LC | UV | MS) detected that the amount of semivolatile and non volatile compounds present in the samples was below 0.01 µg/mL (LOD of the reference standard Irganox 1035).

ICP | MS analysis detected a maximum concentration of the following metal ions: Aluminum 21 ng/mL or 0.063 mg, Barium 0.4 ng/mL or 0.0012 mg, Calcium 160 ng/mL or 0.48 mg, Sodium 100 ng/mL or 0.3 mg, Magnesium 250 ng/mL or 0.75 mg, Titanium 1.1 ng/mL or 0.0033 mg, Zinc 120 ng/mL or 0.36 mg, and Lead 33 ng/mL or 0.099 mg.

7. Appendix

Figure 1

CAD Image Showing the Feed (Yellow), Retentate (Black), and Permeate (Grey) Disposable Flow Kits




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