

# Accelerated Solvent Extraction & Ultra-High Performance Liquid Chromatography Coupled with High-Resolution Mass Spectrometry For Analysis of Additives in Polymers For Biomanufacturing Processes

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## ABSTRACT

To explore the performance of accelerated solvent extraction (ASE) as a technique for the characterization of extractables from single-use bioreactor (SUBs) bags. These plastic assemblies draw concern because they are a potential source of contamination due to extractable and leachable compounds (E&Ls). Characterizing E&Ls from such materials is a necessary step in establishing their suitability for use. Therefore, there is an urgent need for sensitive methods to identify and quantitatively assess compounds in plastic materials. Accelerated solvent extraction (ASE) is a powerful technique that can be reliably used for this purpose.

In this study, Accelerated solvent extraction (ASE) followed by liquid chromatography and Orbitrap-based High-Resolution Accurate Mass (HRAM) mass analysis was found to be an efficient and versatile method for the determination of additives in different multilayer polymer systems from single-use bags. ASE optimization was performed using a design of experiments approach. The type of solvent, temperature, swelling agent addition, static time, and the number of cycles were the selected variables. Optimum conditions were dependent on the type of plastic film. Ethyl acetate and cyclohexane were selected individually as optimum solvents. Optimum temperatures were 90–100 Pressure was set at 1500 psi and extraction time was 30 min in 2 cycles. Swelling agent addition was necessary with polar extraction solvents.

## INTRODUCTION

Single-use technologies (SUTs), in particular single-use bioreactors (SUBs), represent an important improvement in biopharmaceutical manufacturing, due to reduced Requirements for cleaning and sterilization while providing increased sterility assurance, reduced manufacturing turnaround times, and the elimination of cleaning validation and its associated costs.<sup>1-2</sup> SUTs also increase facility flexibility and productivity when compared with traditional stainless steel.<sup>1-2</sup> Other advantages include reduction of manual handling operations and the ability to create totally closed operating systems, reducing bioburden and contamination risk to the process stream.<sup>3</sup> Despite these advantages, SUTs create new challenges in bioprocessing, as various fluids with distinct chemical and physical properties interact with single-use components, producing the extractable (organic and/or inorganic chemical entities) from the plastic material that migrate out of the system and accumulate as leachables.<sup>4</sup> These substances could jeopardize bioprocess performance and cause cell growth inhibition, loss of expensive cell lines, and reduced yields.

## MATERIALS AND METHODS

Square samples for the polymeric film were cut into 0.25 cm<sup>2</sup> squares using scissors. The sample pieces were dispersed in clean Ottawa sand to prevent coalescence during extraction and loaded into a 10 mL stainless steel extraction cell with a glass fiber filter in the bottom cap. Then, the remaining cell volume was filled with clean Ottawa sand and closed with the cell cap. The cell was placed on a Dionex ASE 350 Accelerated Solvent Extractor, which was programmed as indicated in the accelerated solvent extraction conditions table. With help of the sequence editor, multiple methods with different extraction conditions were accomplished in one run. The extracts were evaporated using the Rocket Synergy 2 Evaporator system. One milliliter of acetonitrile was carefully added to reconstitute the dried sample and then it was centrifuged for 30 min at 13,000 x g. The supernatant was placed in a vial for analysis. The selection of the extraction solvent is crucial to optimize factors governing a successful extraction, such as solubility and mass transfer.<sup>5</sup> Solvents recommended for Soxhlet extractions are often also used for ASE.<sup>6</sup> However, as higher temperatures and pressure are used in ASE, care must be taken to avoid polymer dissolution.<sup>5</sup> The ideal situation is one where the extraction is performed at a temperature that causes the maximum swelling without dissolving the polymer.<sup>6</sup> The solubility of polymers can be broadly predicted using solubility parameters, i.e., Hildebrandt parameter,<sup>7</sup> (Table 4) wherein the closer the solubility parameters between the polymer and solvent, the more polymer will dissolve in a solvent at lower temperatures than another solvent with a different solubility parameter

Table 1.

Accelerated solvent extraction conditions			
Extraction cell size	10 mL		
Preheating	5 min		
Pressure	1,500 psi		
Static time	12 min		
Number of cycles	2		
Flush volume	100%		
Purge time	120 s		
Solvent	Hexane		
	Method I	Method II	Method III
Cyclohexane (%)	0	0	5
Temperature (°C)	90	100	90

Reagents and chemicals: Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better filtered through a 0.2 μm filter immediately before use

Table 2.

UHPLC-HRMS conditions	
UHPLC system	Vanquish Flex system
MS detector	Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer
Column	Thermo Scientific™ Accucore™ C18 column, 2.1 x 100 mm, 2.6 μm particle size
Mobile phase	A: 0.1% formic acid, 5 mM ammonium formate, pH 3 B: 5 mM ammonium formate in 90:10 (v/v) acetonitrile/water
Gradient	5% B (0–1.2 min) 5–95% B (1.2–18 min) 95% B (18–26 min) 5–95% B (26.1–29 min) 5% B (29.1–32 min)
Flow rate	0.4 mL/min
Injection volume	5 μL
Column temperature	50 °C, still air mode
Run time	32 min
Mass spectrometric detection	
Ion source	Electrospray ionization (ESI), negative mode
	Sheath gas flow rate: 50 Aux gas flow rate: 10 Sweep gas flow rate: 0 Spray voltage (kV): 3.5 Capillary temp. (°C): 300 S-lens RF level: 60 Aux gas heater temp (°C): 430
Experiments <sup>a</sup>	Full MS/ddMS <sup>2</sup> with inclusion list (Tables 1 and 2)

Operating conditions for UHPLC-HRMS system

Figure 1. Distribution of identified compounds per number of ASE methods, (A); and per type of ASE method, (B)

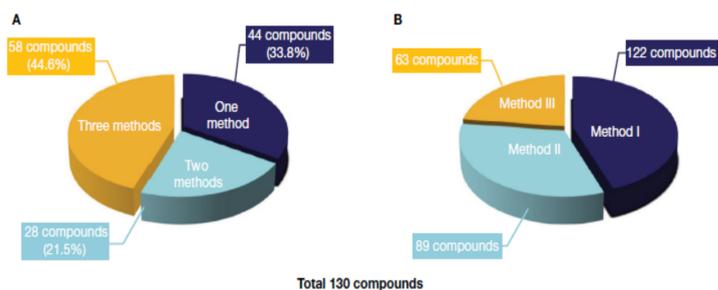


Table 3. Full MS/ddMS<sup>2</sup> inclusion list

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity
621.31045	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> P <sub>2</sub>	+ H	1	Positive
647.45876	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> P	+ H	1	Positive
663.45367	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> P	+ H	1	Positive
205.15979	C <sub>10</sub> H <sub>8</sub> O	- H	1	Negative
475.29717	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> P	+ H	1	Positive
114.09134	C <sub>7</sub> H <sub>8</sub> NO	+ H	1	Positive
277.12818	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub>	+ H	1	Positive
267.17197	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> P	+ H	1	Positive
192.15942	C <sub>11</sub> H <sub>10</sub> NO <sub>2</sub>	+ H	1	Positive
338.34174	C <sub>21</sub> H <sub>20</sub> NO	+ H	1	Positive
431.17878	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S	+ H	1	Positive
225.19614	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O	+ H	1	Positive
250.11862	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	+ H	1	Positive
277.17982	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub>	+ H	1	Positive
219.17434	C <sub>11</sub> H <sub>10</sub> O	+ H	1	Positive
784.52591	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	+ H	1	Positive
531.47717	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub>	+ H	1	Positive

Note: Formula [M] stands for the composition of the active compound, and CS [z] for the charge state of the ion to be fragmented

Table 4. Some identified common additives in plastic films

Formula	Retention time (min)	Mass error (ppm)	Name	CAS	ASE Method (%)	Count			
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	22.37	451.3004	0.08	Oxidized Irgafos 108	1.34	1.02	0.8		
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	3.02	217.0282	0	2-Hydroxy-1,2,3-propanetrifluoromethyl-4-methyl-4-oxo-1,3-dioxane	17.65	0.21	0.19	0.17	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	14.89	267.9720	0	Tetrahydrophthalic anhydride	126.73	2.47	2.31	2.00	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	21.98	268.9470	-1.16	19-(9Z)-hexadec-9-enoic acid (Zigzagene)	119.94	2.83	3.03	3.62	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	22.37	451.3004	-0.25	2,2-Diethyl-1,3-dioxane-5-carboxylic acid (Lumac® C)	119.94	0.09	0.16	0.11	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	22.37	451.3004	0.17	2,2-Diethyl-1,3-dioxane-5-carboxylic acid (Lumac® C)	119.94	0.09	0.16	0.11	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	16.29	217.0282	-0.36	13-(9Z)-hexadec-9-enoic acid (Zigzagene)	119.94	0.07	0.17	0.16	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	14.02	268.9470	0.01	3,3'-Bis(4-tert-butylphenyl)propane (Irganox 1010)	9000	11.9	16.4	16.8	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	21.98	268.9470	0.17	19-(9Z)-hexadec-9-enoic acid (Zigzagene)	119.94	0.4	0.46	0.42	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	15.97	268.9470	-0.18	2,4-Di-tert-butylphenyl ether (DBP)	86.76	0.02	0.01	0.03	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	15.92	475.2972	0.21	1,6-Hexanediol bis(2-ethylhexanoate) (Irganox 1010)	8284	25.1	0.52	0.59	0.27
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	21.98	268.9470	0.45	19-(9Z)-hexadec-9-enoic acid (Zigzagene)	119.94	11.9	16.4	16.8	
C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	21.98	268.9470	0.17	19-(9Z)-hexadec-9-enoic acid (Zigzagene)	119.94	0.4	0.46	0.42	

One hundred thirty compounds were identified from the plastic films by library identity searches using Compound Discoverer 3.2 software, demonstrating the performance and confidence of the applied analytical method for the detection and identification of the extracted compounds

Figure 2. Single-use bioreactor bag



The present work explored the performance of ASE as an extraction technique for characterization of the plastic films (inner layers) from single-use bioreactor bags

Figure 4. Identification of bDtBPP from the plastic film with Full MS and MS/MS spectra

