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Enhanced Monitoring of AAV Downstream Processes Using Liquid Chromatography Approaches

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Introduction

In-process control of AAV downstream processes (DSPs) ensures process efficiency, product quality, safety and consistency [1, 2, 3]. Using liquid chromatography (LC)-based methods such as size exclusion chromatography (SEC), cation exchange (CEX), and anion exchange (AEX) chromatography enables real-time monitoring of critical quality attributes..

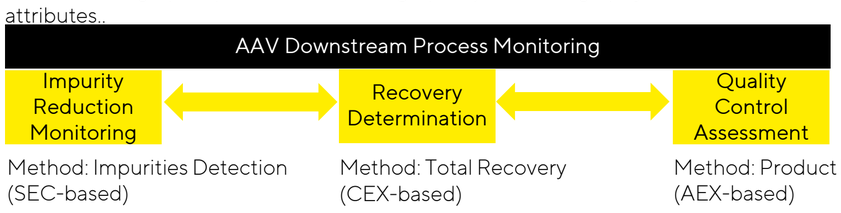


Figure 1: Graphical representation of initial DSP monitoring.

Downstream Process Workflow and Analytics

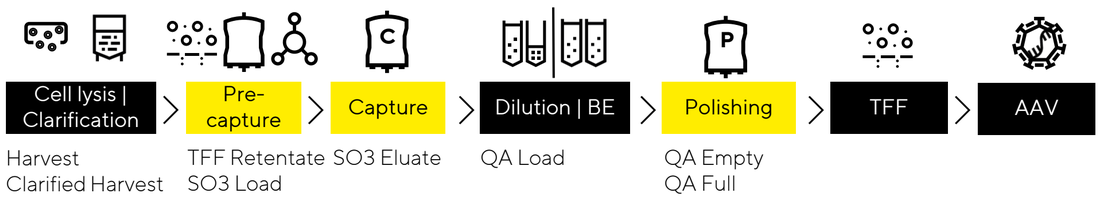


Figure 2: Graphical representation of DSP workflow (DSP steps) and the samples obtained from each step (listed below).

PATfix ® LC-based methods for DSP control are based on the following chromatographic columns:
- BIA SEC Methacrylate Analytical Column with 2000 Å pores (Impurities Detection),
- strong CEX monolithic column – CIMac SO3 0.1 mL analytical column with 2 µm channels (Total Recovery),
- strong AEX monolithic column – CIMac QA HR 0.1 mL analytical column with 2 µm channels (Product Purity).

All columns are provided by Sartorius BIA Separations). Data analysis were performed using PATfix software.

Impurities Detection with SEC-based method

- **Aim**: fingerprinting, impurities identification (aggregates, host cell proteins and DNA) and tracking
- **Background**: separation by size. Broader impurity profiling in a single run, combining multiple signals: UV (260/280 nm), tryptophan/PicoGreen fluorescence (Trp FLD/PG FLD), and multi-angle light scattering (MALS).
- **Case study**: AAV8 DSP impurity reduction. PATfix results were confirmed with orthogonal assays—Bradford total protein (orthogonal to Trp FLD) and PG dsDNA (orthogonal to PG FLD), as summarized in Table 1.

Table 1: Impurity reduction during AAV8 downstream process, as assessed by multiple methods..

Sample name	Trp FLD Area [mVs]	Bradford Assay [µg/mL]	PicoGreen FLD Area [mVs]	PicoGreen Assay [µg/mL]	MALS 90° Area [mVs]
AAV8 Harvest	2465779,0	2584,0	189245,0	4,8	15287,0
AAV8 Clarified Harvest	2296452,0	966,0	170992,0	3,2	3974,0
AAV8 TFF Retentate	9144,0	816,0	33,0	na	686,0
AAV8 SO3 Eluate	7129,0	64,0	5,0	0,1	130,0

Total AAV Recovery Estimation using CEX-based method

- **Aim**: total AAV recovery estimation and optional fingerprinting
- **Background**: separation occurs based on electropositivity. Impurities either pass through the SO3 column (flow-through) or elute during cleaning-in-place (CIP), while the majority of AAV is eluted as a single peak in the salt gradient (Figure 3A). Recovery during DSP is calculated using MALS or Trp FLD signal areas, integrated over the main AAV peak.
- **Case study 1**: applicability to different AAV serotypes (Figure 3B). Most tested serotypes elute in a single peak between 6 and 7 minutes. Notably, only AAV5 eluted earlier at approximately 5 minutes.

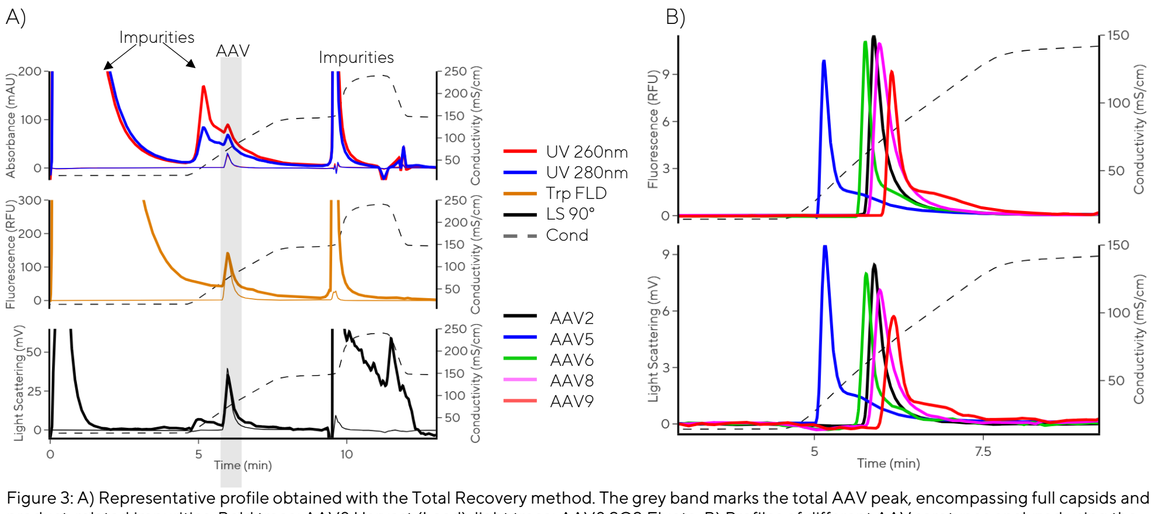


Figure 3: A) Representative profile obtained with the Total Recovery method. The grey band marks the total AAV peak, encompassing full capsids and product-related impurities. Bold trace: AAV8 Harvest (Load); light trace: AAV8 SO3 Eluate. B) Profiles of different AAV serotypes analyzed using the same method.

- **Case study 2**: AAV8 and AAV9 DSPs recovery estimation using PATfix Total Recovery method compared against dPCR and ELISA. PATfix agreed well with both assays (Table 2A and 2B) providing a rapid, serotype-agnostic readout without separate specific kits. MALS aligned more closely with dPCR and ELISA than Trp FLD across serotypes.

Table 2: Recovery of A) AAV8 and B) AAV9 DSP samples. Percent recovery was calculated by LS90° area, Trp-FLD area, dPCR, and ELISA. *dPCR detects only encapsidated genomes and cannot assess recovery of empty/full-enriched samples. **Not assessed.

A)

DSP Sample	Recovery [%]			
	dPCR	MALS Area	FLD Area	ELISA
Clarified Harvest	109	87	96	132
TFF Retentate	121	116	134	157
SO3 Load	78	78	88	133
SO3 Eluate	52	52	59	43
QA Load	50	49	55	88
QA Empty	3*	24	35	25
QA Full	43*	26	21	23

B)

DSP Sample	Recovery [%]			
	dPCR	MALS Area	FLD Area	ELISA
Clarified Harvest	94	126	165	na**
TFF Retentate	89	73	128	na**
SO3 Load	99	107	139	na**
SO3 Eluate	67	77	101	na**

Product Purity Assessment with AEX-based method

- **Aim**: product purity assessment → enabling safer AAV gene therapies
- **Background**: separation of full AAV8 capsids from the other AAV8 species by their electronegativity.
- **Case study**: %F were measured in four purified AAV8 samples. Results from the PATfix Product Purity (PP) method (Figure 4, Table 3A) were compared with mass photometry (MP) and PATfix ultracentrifugation (UC). Results aligned closely with UC (RSD ≤3%; Table 3B), while MP showed greater variability.

Table 3: A) Percentage of full AAV8 capsids (%F) determined using PP, UC and MP; and B) Relative standard deviation (%RSD) for each method, used to evaluate measurement accuracy across the tested samples.

A)

DSP Sample	Full AAV Determined [%]		
	PP	UC	MP
AAV8 purified sample 1	93.6	93.5	88.1
AAV8 purified sample 2	68.4	67.9	62.8
AAV8 purified sample 3	80.8	77.5	78.3
AAV8 purified sample 4	92.3	90.4	81.7

B)

DSP Sample	RSD [%]		
	PP vs UC	UC vs MP	PP vs MP
AAV8 purified sample 1	0.1	4.3	4.2
AAV8 purified sample 2	0.5	6.0	5.5
AAV8 purified sample 3	3.0	2.2	0.8
AAV8 purified sample 4	1.4	8.6	7.2

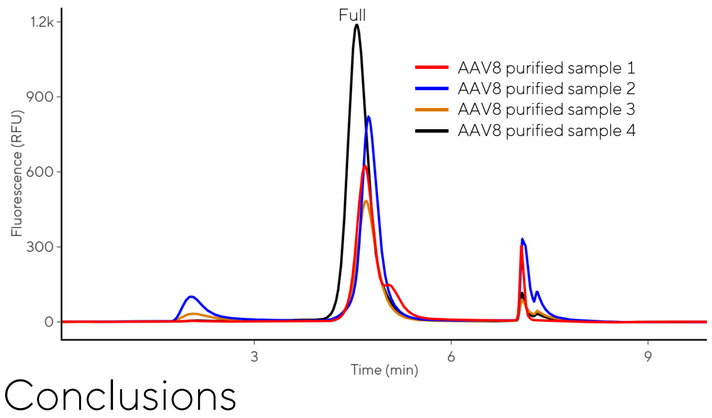
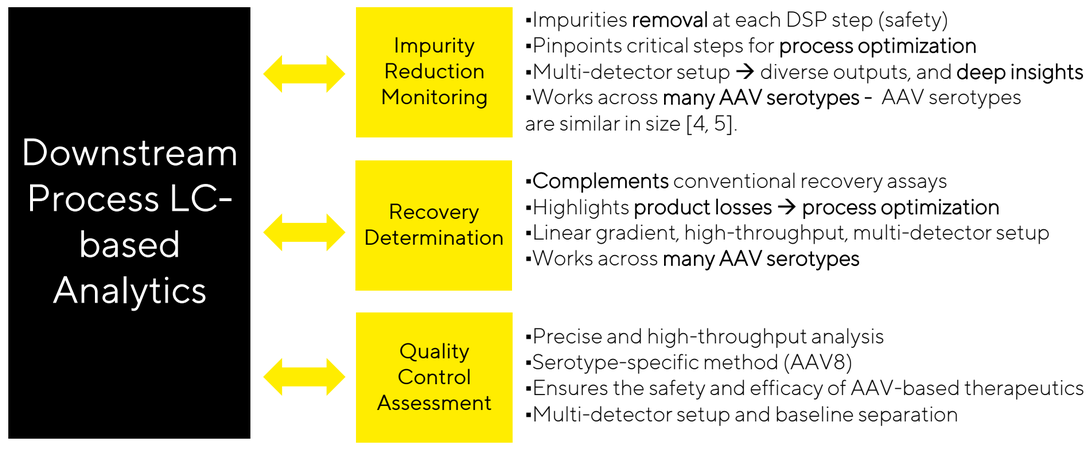


Figure 4 : Various AAV8 purified samples analyzed by the Product Purity method. Sample 1 – 4.

Conclusions



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