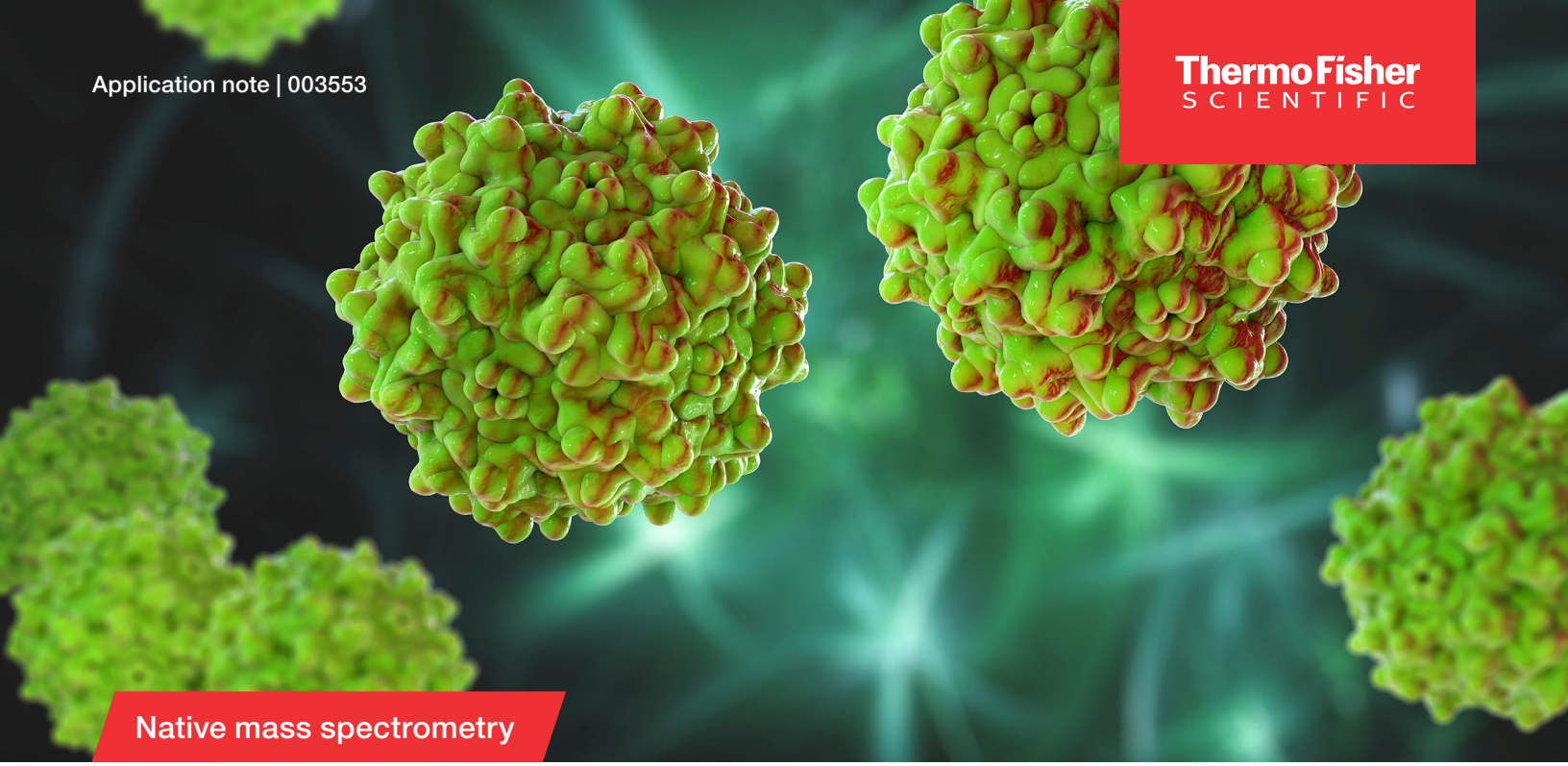

Application note | 003553

Native mass spectrometry

Virus molecular weight and empty/full capsid ratio measurements on a Q Exactive UHMR mass spectrometer using Direct Mass Technology mode

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Keywords

Adeno-associated virus (AAV), Q Exactive UHMR mass spectrometer, Direct Mass Technology, charge detection mass spectrometry, Orbitrap technology

Application benefits

- Intact adeno-associated virus (AAV) characterization using a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer
- Rapid molecular weight (MW) and ratio assessment of empty and full AAV particles using Direct Mass Technology

Goals

- Demonstrate the precise measurement of AAV MW and ratio on a Q Exactive UHMR MS
- Recommend proper instrument and STORIboard parameters to accurately and reproducibly determine AAV empty/full ratios

Introduction

AAVs are widely recognized as safe and effective vectors for gene therapy, capable of delivering genes to specific tissues and cells.[1] A critical step in the successful application of AAV-based therapies is the precise quantification of the percentage of capsids containing the desired genome. Traditionally, analytical ultracentrifugation (AUC) has been employed to determine full-to-empty AAV capsid ratios, though this method requires significant sample volumes (>100 µL at titers of ≈10^{12} vg/mL).[2]

In contrast, Thermo Scientific™ Orbitrap™-based Charge Detection Mass Spectrometry (CDMS), also known as Direct Mass Technology mode, offers the advantage of delivering full-to-empty AAV ratios within minutes, consuming less sample than conventional methods such as AUC.[3] This report outlines the optimal instrument settings for AAV measurements to ensure accurate and reproducible MW and ratio determinations.

Experimental

Sample preparation

Empty (2E+13 vp/mL) and full (CMV-GFP, 2E+13 vg/mL) AAV2, AAV8, and AAV9 capsids were purchased from Virovek (Hayward, CA). The empty and full capsids were mixed in a volumetric ratio of 25/75, 50/50, and 75/25, respectively, and buffer exchanged into 200 mM ammonium acetate using a 100 kDa MW cutoff filter (Millipore UFC510008) to produce samples containing v/v 25%, 50%, and 75% empty capsids.

Methods

Empty and full AAV mixed to known ratios were measured with a variety of instrument parameters listed in Table 1 on a Q Exactive UHMR MS with Direct Mass Technology mode. Nitrogen was used as the trapping gas. Heavy gases such as xenon or sulfur hexafluoride (SF_6) are recommended when laboratory conditions permit. All ions were produced by electrospray ionization using Thermo Scientific™ borosilicate emitters (P/N ES387/388) and a spray voltage of ≈1.2 kV in a Thermo Scientific™ NanoSpray Flex™ ion source. Each acquisition required 1-2 min. Empty/full ratios were determined using the STORIboard analysis package build 1.0.24204.1 (Proteinaceous).

Table 1. Q Exactive UHMR MS settings

Parameter	Range [min, max]
Ion transfer tube temp. (C)	[250, 350]
Source DC offset (V)	[-21, 30]
In-source trapping (desolvation) (V)	[-100, -1]
Injection flatapole (V)	[1, 10]
Injection flatapole RF (V)	[250, 700]
Interflatapole lens (V)	[1, 8]
Bent flatapole (V)	[1, 7]
Extended trapping (eV)	[5, 300]
Trapping gas (N_2, mbar)	[6.2e-11, 4.9e-10]
HCD purge time (ms)	[5, 50]
HCD field gradient	[20, 100]
HCD RF (V)	[250, 900]

Data processing

RAW files were processed using the latest version of the data analysis software STORIboard, resulting in masses (MDa) and percentages for empty, partial-filled, full, and over-filled capsids. The data here are presented as a fraction of empty capsids, as empty capsids are simpler to distinguish from filled, partially filled, and over-filled capsids. STORIboard processing parameters are listed in Table 2. STORIboard automatically calculates the molecular weight and percentage of each AAV capsid distribution. Figure 1 shows the example of STORIboard processing results.

Table 2. STORIboard processing parameters

STORI processor parameters	
R^2 threshold	0.99
Duration threshold	0.1
Minimum time of death	0.2
Minimum time of birth	0.1
Signal-to-noise threshold	1
Apply frequency correction	True
Charge assignment parameters (Central limit)	
Number averaged ions	1
m/z tolerance	50

Results and discussion

Impact of Q Exactive UHMR MS parameters on ratio calculation

As shown in Figure 2, many of the parameters have minimal impact on the measured AAV empty/full ratios. For example, it has been widely believed that heavier gases, such as SF_6 or xenon were necessary to properly trap AAV capsids in the HCD cell. However, the data presented indicate that accurate AAV empty/full ratios can be measured using relatively low nitrogen pressures in the HCD cell by collecting enough ions for statistical relevant measurement. In contrast, two parameters, in-source trapping (desolvation) voltage and injection flatapole RF voltage, stand out as significantly biasing against full capsids. Figure 3 highlights how drastically the measured full/empty ratio changes with a modest increase in desolvation voltage.

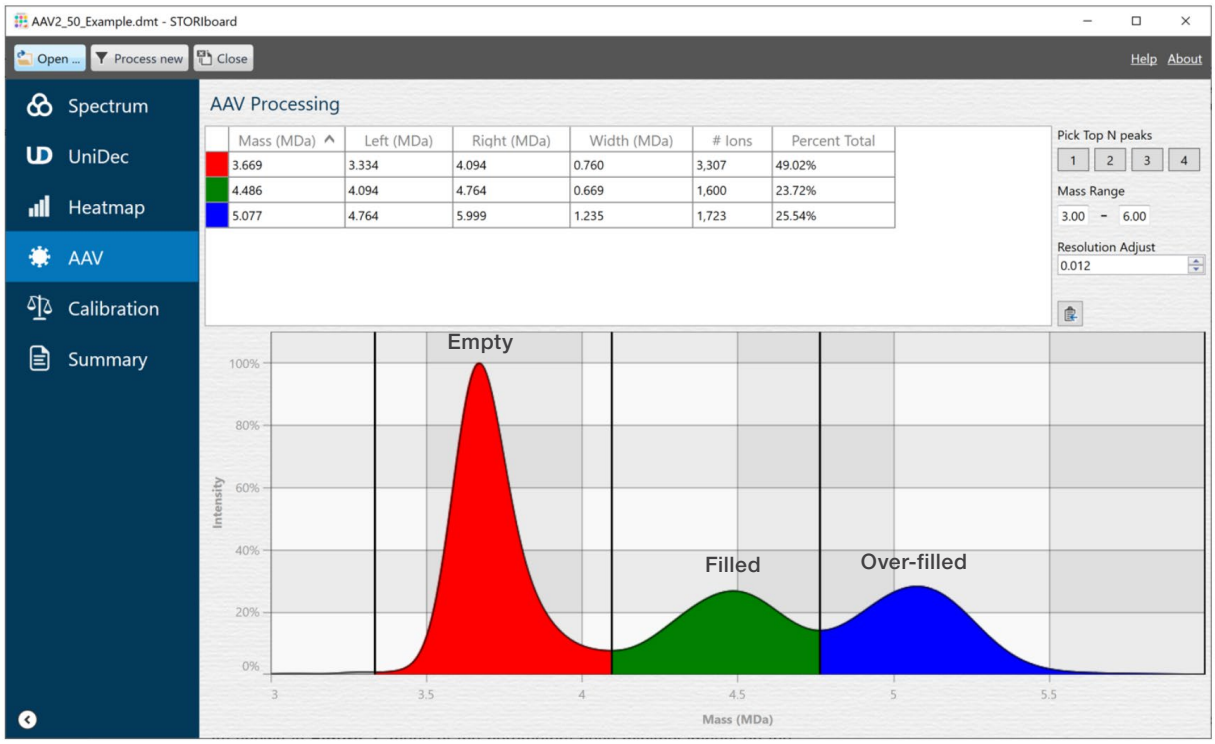

Figure 1. STORIboard analysis results for a 50% empty AAV2 sample (empty, filled, over-filled labels added)

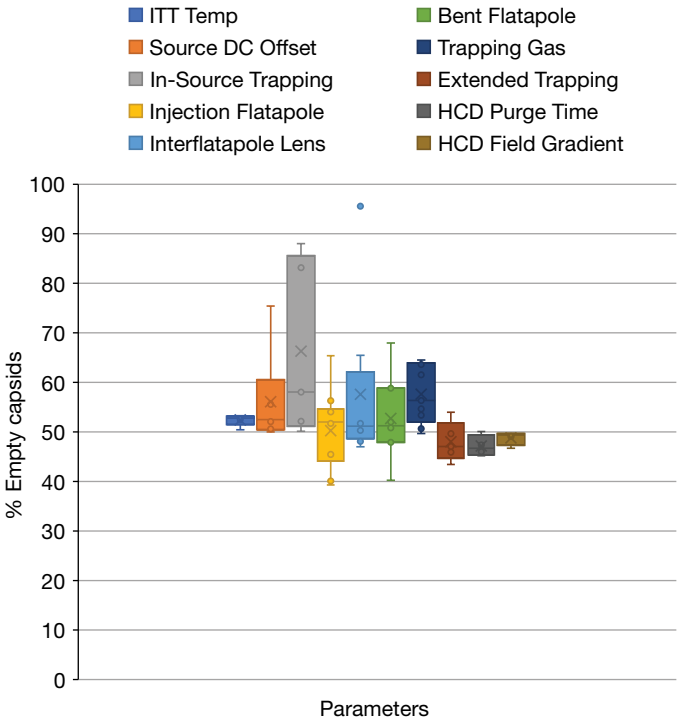

Figure 2. The impact of individual instrument parameters on measured AAV ratios for 50% empty AAV2 based on the instrument parameters listed in Table 1. The larger the box, the more sensitive the measured ratio to the parameter setting.

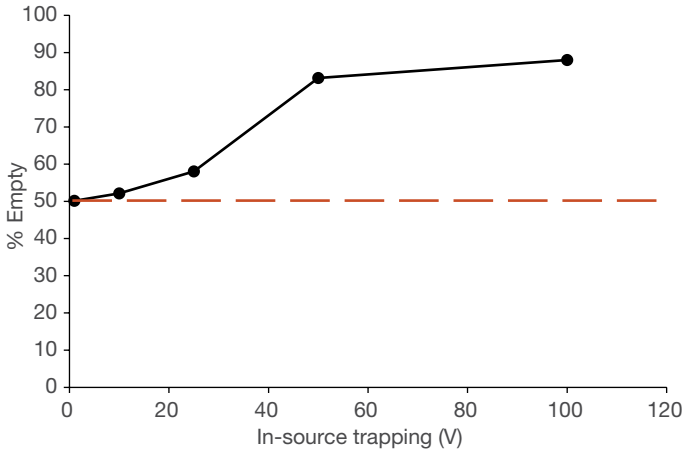

Figure 3. Percent empty capsids versus in-source trapping voltage for 50% empty AAV2

A possible explanation is that at higher injection energies, it is more difficult to retain ions in the radial dimension. For higher *m/z* ions, this effect is more pronounced. In general, RF voltages on the injection flatapole radially confine the ions, allowing for better desolvation during in-source trapping. Higher voltages allow for better confinement of higher *m/z* ions.[4]

However, the default injection flatapole RF voltage at high *m/z* transmission is the maximum that can be applied. So, as a test, the injection flatapole RF voltage was lowered, and the individual rates of empty and full AAV ions (average ions/spectrum) were measured as a function of in-source trapping (desolvation) voltage. The results are presented in Figure 4. The rate decreases for all ions as in-source trapping is increased. At the maximum injection flatapole voltage, the rate decreases more rapidly for full AAV ions. Lowering the injection flatapole RF voltage results in a significant difference in average ions per spectrum between the empty and full ions, even at low in-source trapping voltages. These findings are consistent with the original assumption that full ions are being lost radially with more in-source trapping.

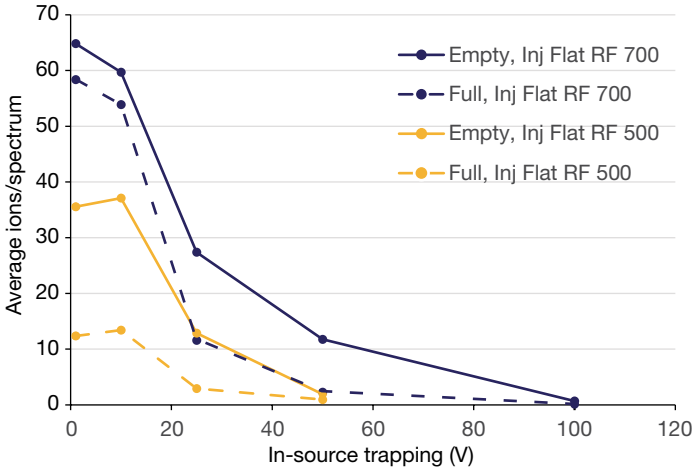

Figure 4. Rate (average ions/spectrum) of empty and full-plus-overfilled capsids versus in-source trapping voltage for 50% empty AAV2 at different injection flatapole RF voltages

As a result, desolvation should be performed mainly in the HCD cell. Extended trapping, trapping gas setting, HCD purge time, and HCD field gradient have all shown minimal impact to the measured AAV full/empty ratios. Furthermore, recent improvements in signal processing have demonstrated the ability to account for incomplete desolvation when measuring heavier species.[5]

Although certain parameters may not impact the measured full/empty ratio, they may result in lower sensitivity. Examples include the trapping gas setting and source DC offset, as shown in Figure 5. Parameters that demonstrate *m/z* dependence, such as the source DC offset and trapping gas setting, may have different sensitivity even if the full/empty ratio remains constant. This is shown by the average ions per spectrum for a sample with reasonably stable electrospray conditions. For example, Figure 5 gives the percent empty capsids and average ions per spectrum as the source DC offset is varied. From this data, it is recommended to set source DC offset = 0 for an accurate full/ratio measurement with the best sensitivity.

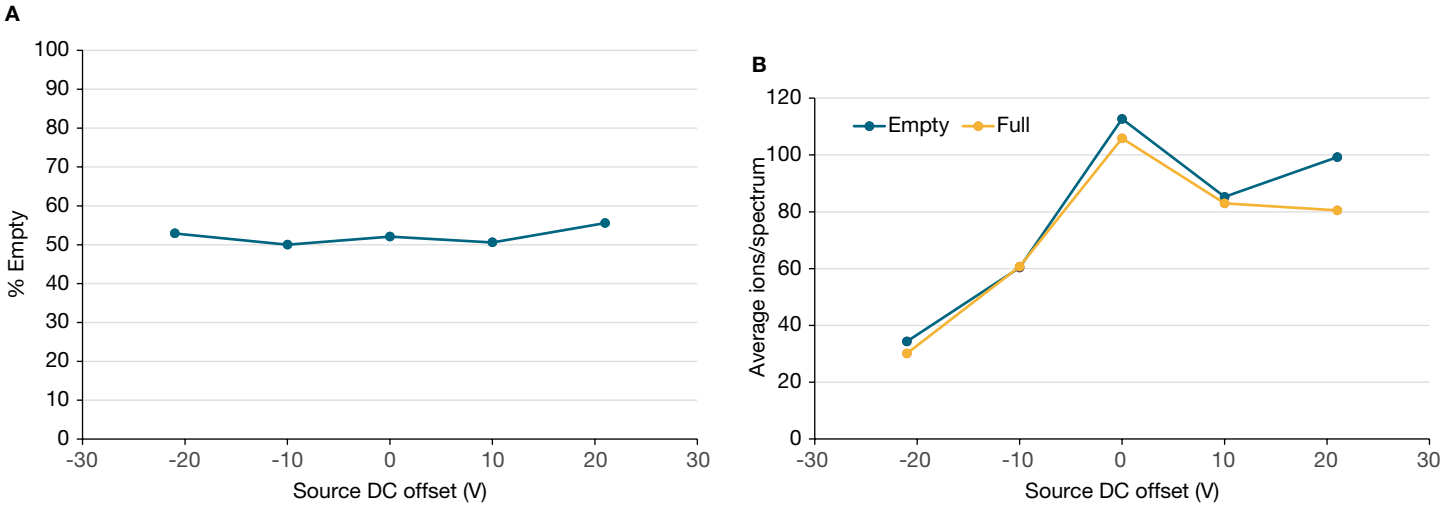

Figure 5. (A) Percent empty capsids versus a select range of source DC offset voltages and (B) the associated average empty and full-plus-overfilled capsid ions per spectrum for 50% empty AAV2

AAV ratio reproducibility

To test the validity of the suggested parameters for accurate full/empty capsid ratio determination, replicate measurements were made on three different AAV serotypes (AAV2, AAV8, and AAV9) and in volumetric mixtures (25%, 50%, 75% empty), as shown in Figures 6A and 6B. A minimum of five replicates were performed for each condition. Figure 6C highlights the reproducibility of 50% AAV8 empty capsids measured versus trapping gas setting for three different instruments.

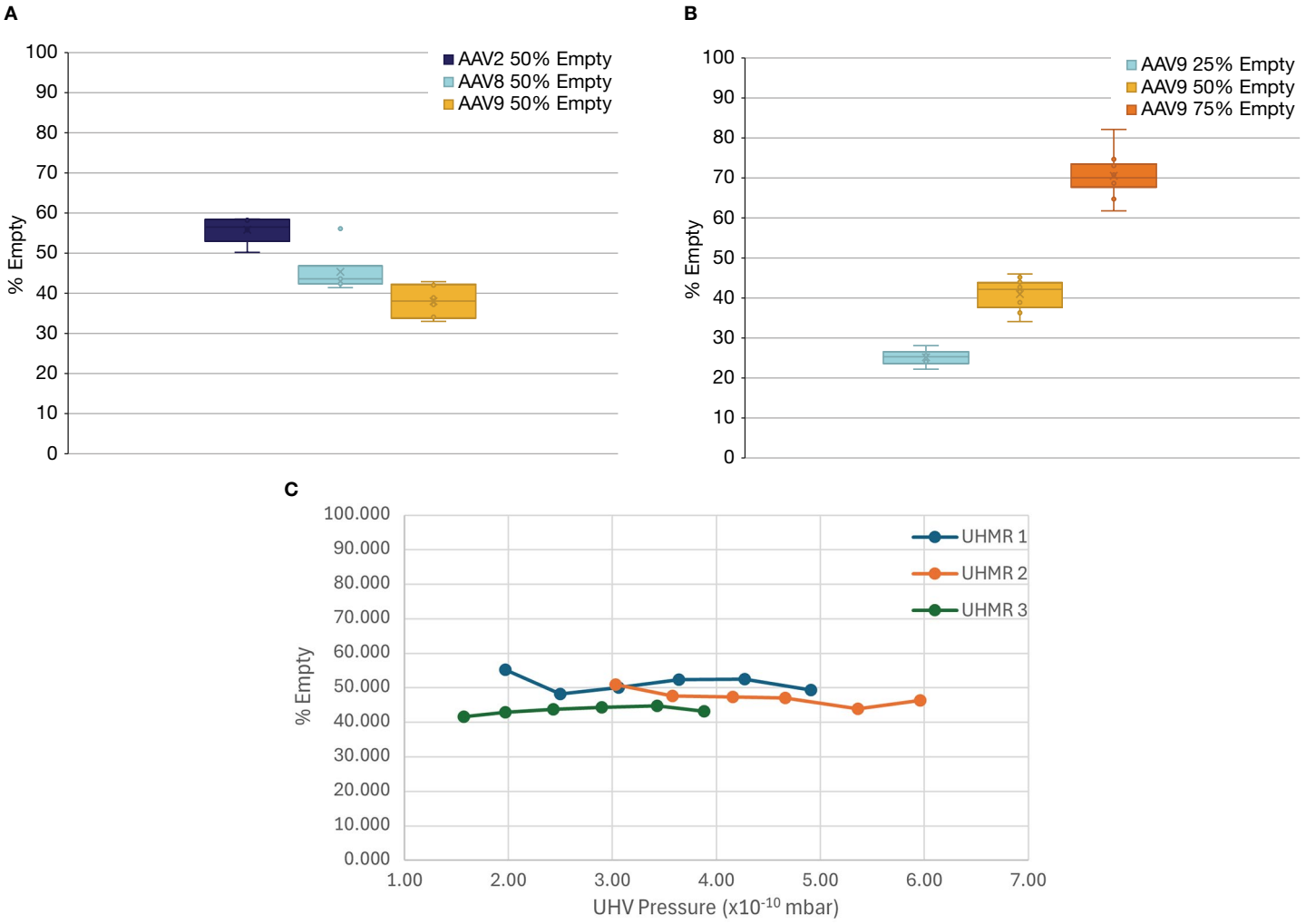

Figure 6. Full/empty capsid ratio measurement (A) by serotype; (B) by volumetric ratio; (C) by instrument

Conclusions

Of the many adjustable parameters on the Q Exactive UHMR MS, a select few, including in-source trapping, demonstrate an impact on the measured AAV full/empty ratios. However, these deviations are largely predictable for high *m/z* ions and can be avoided by optimizing the set of parameters listed in Table 1. From the optimized settings shown in Table 3, a range of full/empty ratios for any AAV serotype can be measured accurately and reproducibly.

Table 3. Recommended parameters for AAV measurement on the Q Exactive UHMR MS

Parameter	Value
Ion transfer tube temp. (C)	350
Source DC offset (V)	0
In-source trapping (V)	-10
Injection flatapole (V)	4
Interflatapole lens (V)	3
Bent flatapole (V)	2
Extended (HCD) trapping (eV)	5
Trapping gas (x10^{-10} mbar)	≈5.0
HCD purge time (ms)	15
HCD field gradient	65

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