Simplifying Progress

PATfix® Analytical Method for Orf Virus Sample Characterization

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Introduction

In recent years, the Orf virus (ORFV) has become a promising tool for protective recombinant vaccines and oncolytic therapy (1-3). Regardless of the high potential for use and the broad range of possible applications, enhancing the purity of the final product, and consequentially, decreasing the negative impacts attributed to impurities is essential. Accurate and comprehensive analytical data plays an important role in process development. However, analytical methods available for characterising ORFV components at various phases of production, including upstream and downstream processes are rather limited.

For this purpose, we developed a high-performance liquid chromatography (HPLC) PATfix analytical method, based on multiple-detector PATfix technology coupled with CIMac QA-0.1 (6 mm) analytical column, enabling us to assess sample composition at various stages of ORFV production and determination of ORFV particles.

1. PATfix analytical platform setup

In-process samples were analyzed using PATfix HPLC system and anion-exchange (AEX) chromatography on CIMac QA-0.1 (6 mm) monolithic analytical column (Figure 1). In a preliminary experiment, different conditions were tested – various CIMac analytical columns, composition of buffers, pH and length of the elution gradient (data not shown). The ORFV was eluted in a linear salt gradient. The flow rate was 1 mL/min. The running buffer was 20 mM BTP, 1 % sorbitol and 0.1 % poloxamer 188; pH 7.0. Elution was performed in 20 column volumes using elution buffer with the following composition: 20 mM BTP, 2 M NaCl, 1 % sorbitol, and 0.1 % poloxamer 188; pH 7.0. The PATfix HPLC system with integrated UV-Vis detector, conductivity, and pH monitoring was additionally equipped with two dual-wavelength fluorescence detectors and a multi-angle light scattering MALS detector. This multiple-detector PATfix technology enabled simultaneous detection of absorbance at 260 nm and 280 nm, fluorescence, set to Ex: 485 nm and Em: 520 nm wavelengths for PicoGreen® detection and Ex: 280 nm and Em: 348 nm for intrinsic tryptophan fluorescence detection, and light scattering emitted/scattered from the sample (90° angle). To confirm the retention time of DNA impurities, we analysed standard DNA solutions. For this purpose, we injected 50 ng of standard sss DNA and standard λ DNA to the column with the same PATfix analytical method.

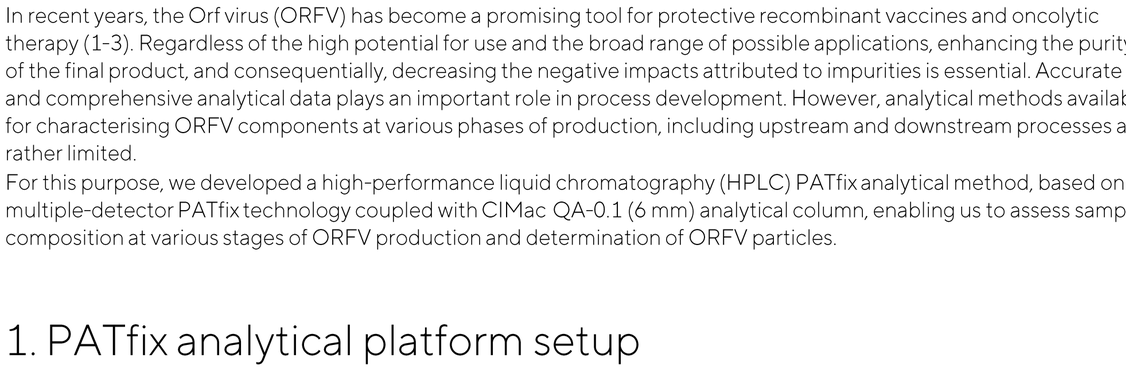

Figure 1: Scheme of PATfix analytical platform setup

2. Sample preparation

Four different elution samples from the chromatographic purification process (Figure 2) and the ORFV starting material were included in the study. Virus material was purified in-house and analysed with dPCR and infectivity assay to determine the virus concentration in the sample.

To achieve suitable pH and conductivity of the sample and so provide proper binding conditions, samples were diluted with running buffer. For PicoGreen® fluorescence analysis, Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen) was added to each sample and incubated for at least 2 hours at 4°C in the dark.

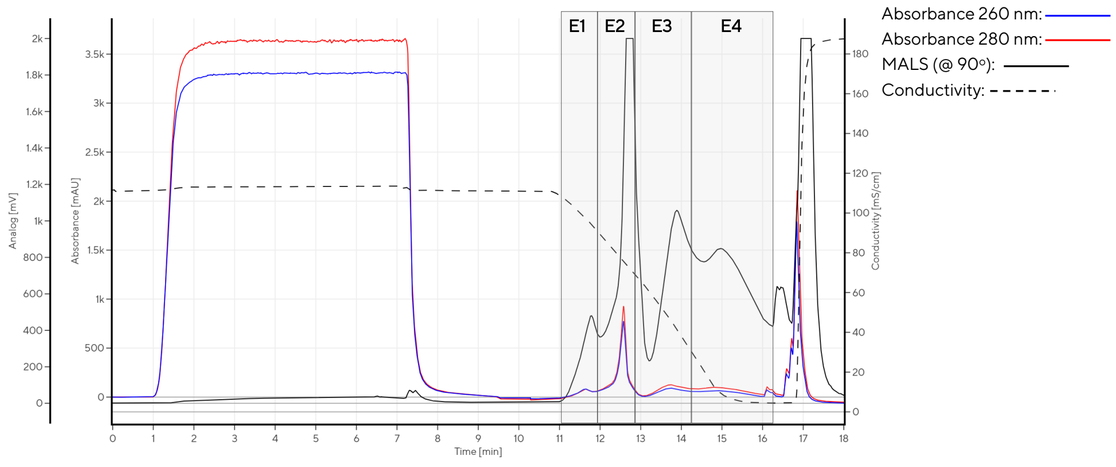

Figure 2: Preparative chromatogram of in-house chromatographic purification: Elution 1 – E1, Elution 2– E2, Elution 3 – E3 and Elution 4 – E4

Sample	dPCR analysis (vg/mL)	Infectivity analysis (IFU/mL)	MALS area[a] (mV.s)
ORFV starting material	2.88E+09	1.61E+08	51 071
E1	1.19E+07	ND[b]	1 194
E2	1.82E+09	1.36E+08	25 469
E3	9.70E+08	1.03E+07	10 861
E4	NA[c]	5.83E+07	19 313

Table 1: ORFV concentration determined with dPCR and infectivity assay, and MALS signal area values from PATfix analytical chromatograms
[a] Signal values were adjusted considering the dilution factor; [b] Not detected; [c] Not assessed

3. Results – sample characterization and determination of ORFV particles

The PATfix analytical method allows tracking the ORFV throughout the process and simultaneously monitoring impurity elimination (Figure 3). In addition to absorbance at 280 nm and 260 nm, we recorded the intrinsic tryptophan (TRY) fluorescence and PicoGreen® (PG) fluorescence for screening the level of protein and DNA impurities, respectively. A MALS detector was also employed (90° angle) to monitor the ORFV. The ORFV product is visible with peak alignment of all signals: UV 260 nm and 280 nm, MALS, PicoGreen®, and tryptophan fluorescence at a retention time of 4.9 min, when the signal is not saturated. MALS area signals were compared to the concentration of ORFV determined with dPCR and infectivity assay (Table 1). Our results confirm the orthogonality between the MALS signal and dPCR and infectivity assay values (Figure 4 (A), (B)). With the PicoGreen® fluorescence signal, we determined the retention time of DNA impurities (Figure 4 (C)).

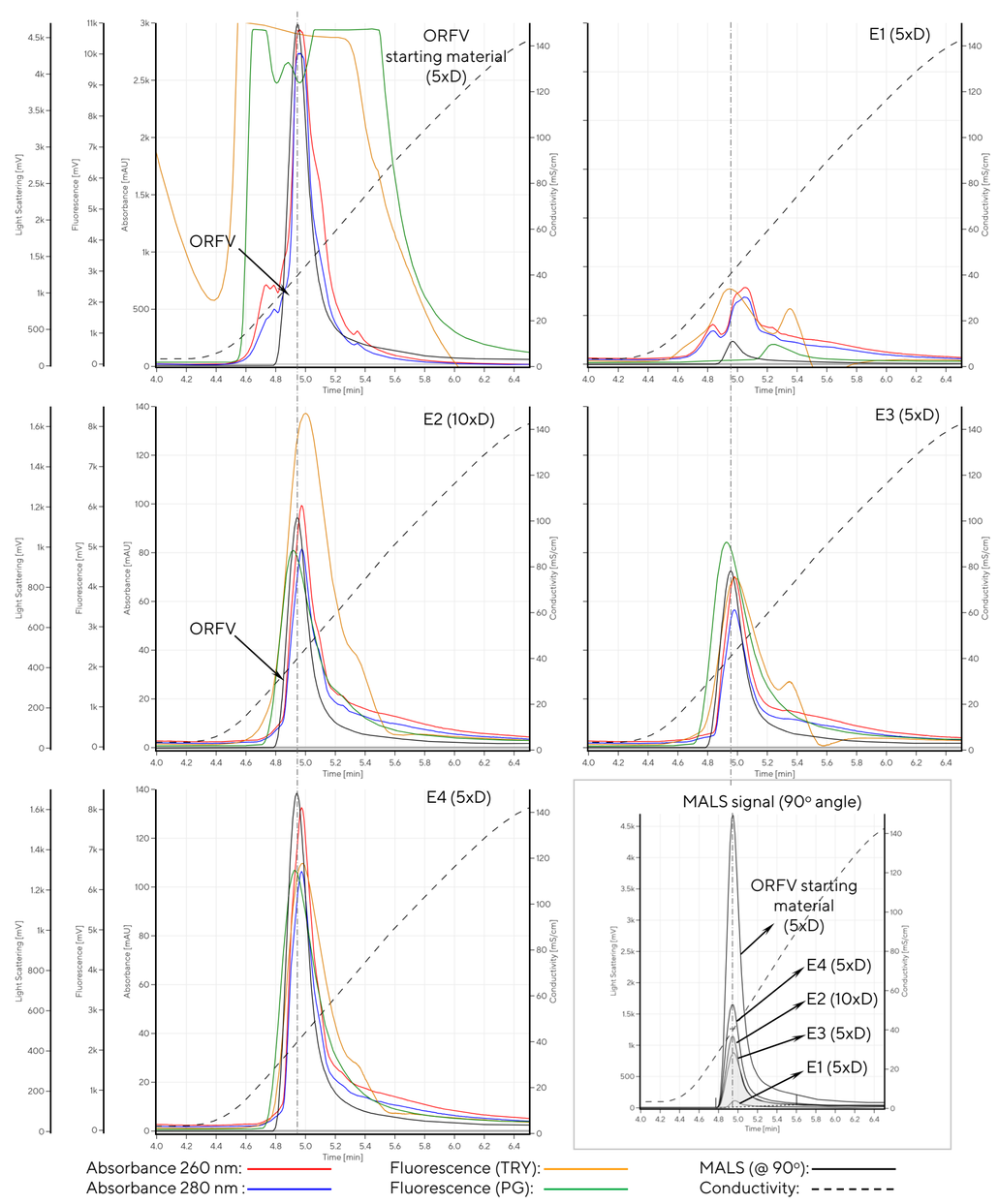

Figure 3: PATfix analytical chromatograms of in-process samples: Elution 1 – E1 (5 x diluted), Elution 2 – E2 (10 x diluted), Elution 3 – E3 (5 x diluted), Elution 4 – E4 (5 x diluted) and ORFV starting material (5 x diluted)

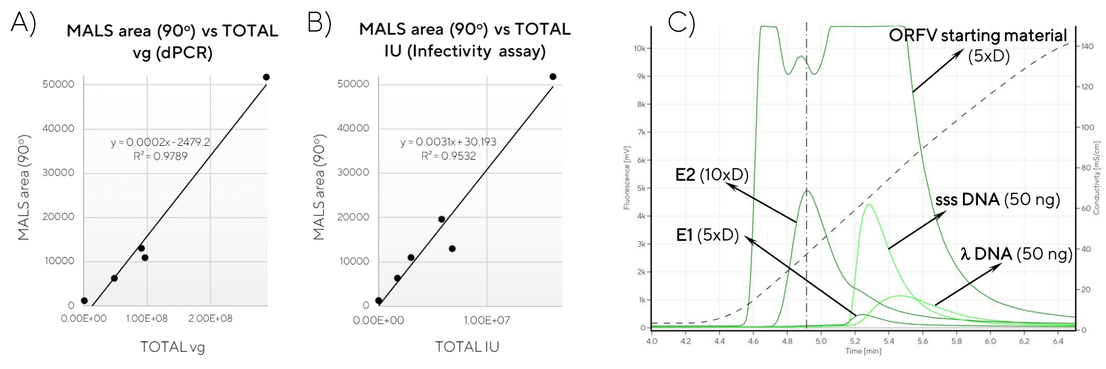

Figure 4: (A) MALS area signals (90° angle) vs dPCR and (B) infectivity assay. (C) PicoGreen® fluorescence signals of two chromatographic elution samples (E1 and E2) and ORFV starting material and two standard DNA solutions.

6. Conclusion

The rapid PATfix analytical method presented here detects ORFV product and records impurity profiles simultaneously, making it suitable for ORFV samples of various complexities. Consequently, it could be a useful tool for monitoring ORFV production and purification processes.

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References

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