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Production scale up of SDVB monolithic columns for the purification of mRNA molecules

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Introduction

Messenger RNA (mRNA) is becoming a major contributor in the fields of **gene therapy** and **vaccines**, including those developed in response to the **COVID-19** pandemic. Convective Interaction Media® (CIM®) **Styrene-divinylbenzene** (SDVB) monolithic columns are promising for high resolution purification and separation of mRNA, enabling large-scale production of this molecule.

This study demonstrates the ability to prepare homogeneous **SDVB monoliths** with desired chromatographic properties and economical analytics over the whole size range.

1. Experimental approach

SDVB monoliths were prepared by **free-radical thermal polymerization** of styrene and divinylbenzene under isothermal reaction conditions. The desired pore-size distribution was achieved using **conventional porogenic solvents**. The prepared SDVB columns exhibited high degree of crosslinking with desired morphology. A well-controlled radical polymerization process was transferred from the laboratory to **industrial scale**, i.e., from 1 mL columns up to 8000 mL **CIMmultus™ columns**. Furthermore, the production of SDVB monoliths was scaled down to 0.1 mL CIMac™ columns, demonstrating our ability to control the polymerization process over the whole size range.

2. Gating Strategy

Another challenge in this study was to **develop inline analytics** that could confirm monolith homogeneity and desired chromatographic properties for columns from 0.1 to 8000 mL. For this purpose, two HPLC chromatographic methods were used. The **cGMP compliant** is an **isocratic one**, separating unretained uracil and retained small molecule, while the **bioapplication-based** employs size separation of **two messenger RNA molecules** in an **acetonitrile gradient**.

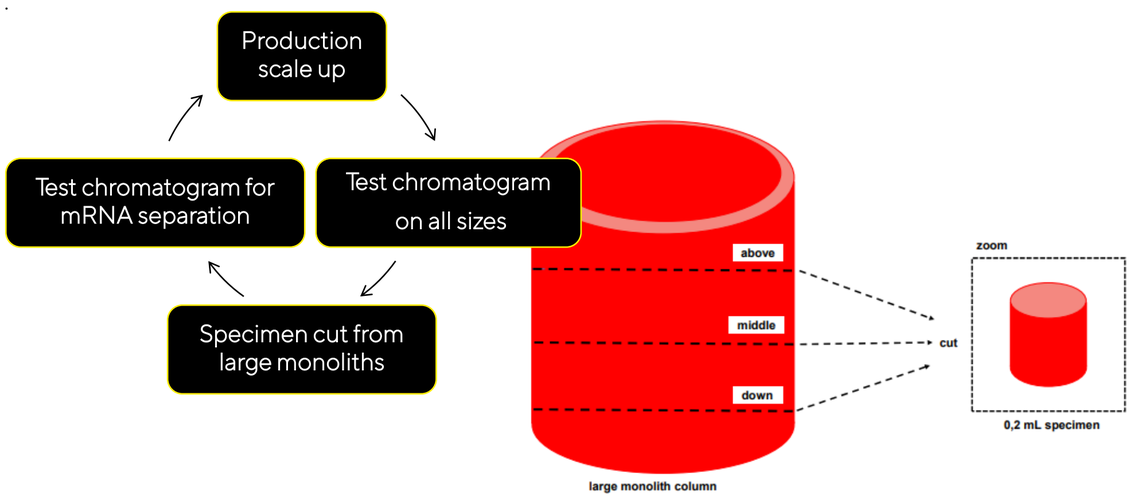


Figure 1: Diagram of gating strategy of production scale up and corellation between cGMP compliant and bioapplication-based method

3. Results – Method for Test Chromatogram

An **isocratic liquid chromatographic method** with UV detection at 270 nm was used for standard test chromatogram **(TK)** to verify packaging conditions and column performance. Mobile phase consisted of a mixture of ethanol and salt. The sample used consisted of an unretained uracil and a retained small molecule. The loop volume and flow rate were set according to column size. To eliminate the influence of different HPLC systems two chromatographic parameters were considered, i.e., **retention volume** of HPLC system with column **(V_r)** and **normalized retention factor** of sample **(k`)**.

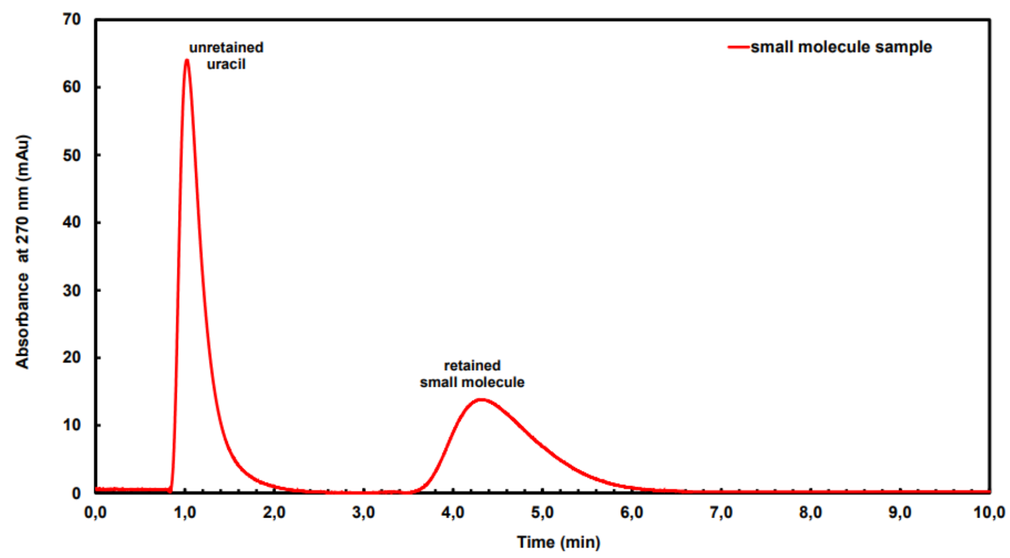


Figure 2: Typical test chromatogram of 80 mL column with retained small molecule and unretained uracil for monitoring chromatographic properties and repeatability of produced SDVB columns.

4. Results – Method for mRNA molecules

The gradient separation method with acetonitrile was used to test performance of 0,2 mL columns, which were cut from the large monolith. The normalized retention factors of two mRNA molecules ($K_{1000\ nt}$ and $K_{4000\ nt}$) were calculated.

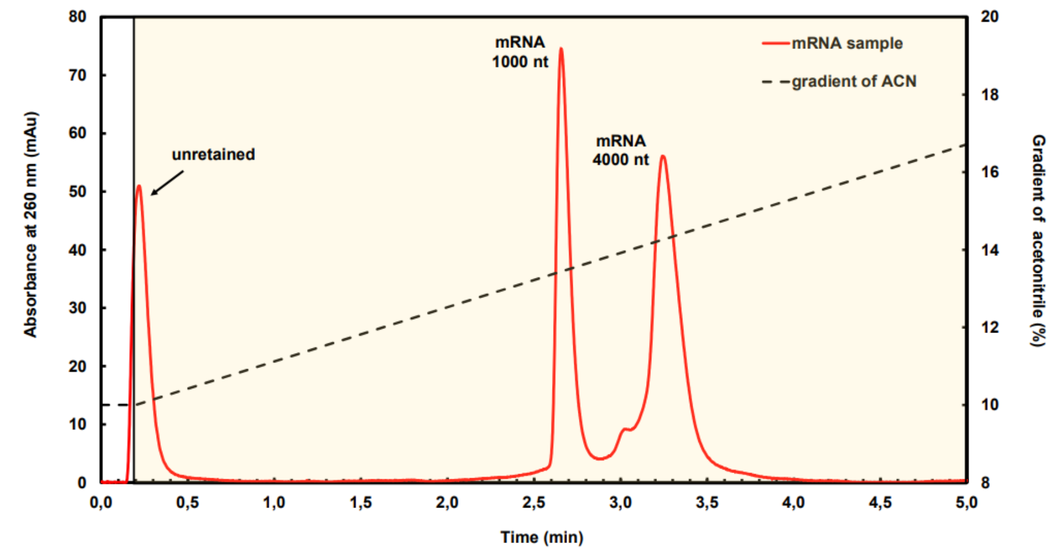


Figure 3: Representative mRNA chromatogram on 0,2 mL specimen of two mRNA molecules

5. Results – Correlation between TK and mRNA

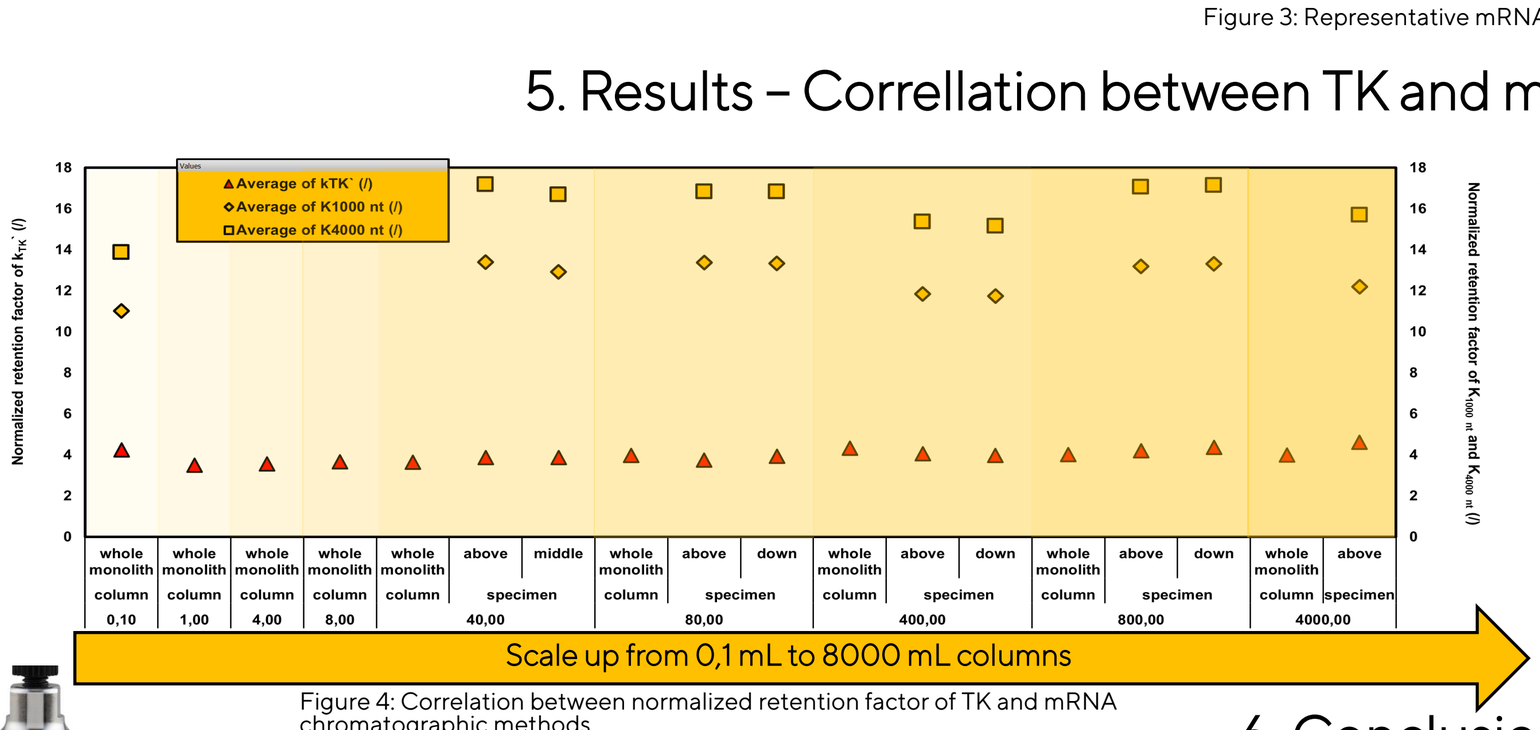
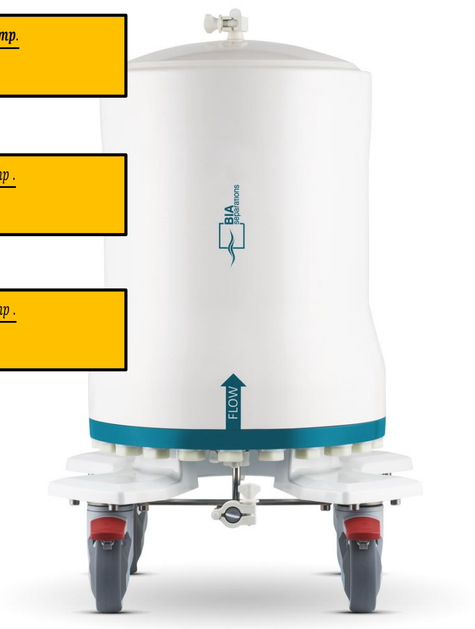


Figure 4: Correlation between normalized retention factor of TK and mRNA chromatographic methods.

$$k` = \frac{t_{retained\ comp.} - t_{unretained\ comp.}}{t_{unretained\ comp.} - t_{SST}}$$

$$K_{1000\ nt} = \frac{t_{1000\ nt} - t_{unretained\ \ comp.}}{t_{unretained\ \ comp.}}$$

$$K_{4000\ nt} = \frac{t_{4000\ nt} - t_{unretained\ \ comp.}}{t_{unretained\ \ comp.}}$$



6. Conclusion

- Correlation between cGMP and bioapplication-based mathods
- Scale up from 0,1 mL to 8000 mL columns
- highly reproducible