

Monitoring mRNA production using CIMac™ Oligo dT analytics

Ana Ferjančič Budihna*, Anže Martinčič Celjar, Blaž Bakalar, Nina Mencin, Janja Skok, Rok Sekirnik, Andreja Gramc Livk, Aleš Štrancar

¹ BIA Separations, part of Sartorius, Mirce 21, SI-5270 Ajdovščina, Slovenia

* Corresponding author: Ana.F.Budihna@biaseparations.com

Introduction

mRNA has been at the forefront of both scientific and general public interests from the start of the COVID-19 pandemic. The demand for the mRNA product has been incredible for the last couple of years. However, there are still limited options available for a rapid mRNA quantification and characterization. In this work, mRNA analytics using a CIMac™ Oligo dT column is presented.

mRNA is a specialized group of RNAs that carries the blueprints for building proteins from the cell's DNA in the nucleus to the ribosomes in the cytoplasm. One of the features of mRNA molecules is a polyadenylated (poly(A)) tail on the 3' end, that can be up to 250 nucleotides long. This feature enables mRNA to bind to the Oligo dT column.

HPLC Oligo dT analytics provide a solution for fast and reproducible quantification of mRNA throughout all the process steps of mRNA production and purification. The presented method was validated using mFix4, an uncapped mRNA analog produced in-house, 3969 nt long molecule with a poly(A) tail length of 95 nucleotides.

1. Experimental approach

A-T base pairing between dT oligonucleotide and poly(A) tail of the mRNA is promoted at physiological pH and high salt (500 mM) conditions, where electrostatic repulsion between negatively charged mRNA and Oligo dT backbones is neutralized. Species without a poly(A) tail do not bind to the column and are eluted in a flow through (FT). Elution is performed at low ionic strength and high pH by breaking A-T base pairs. A PATfix HPLC analytical system with UV 260 nm detector, pH, and conductivity monitors was used for all analytics. Monitoring signals were analyzed with PATfix software.

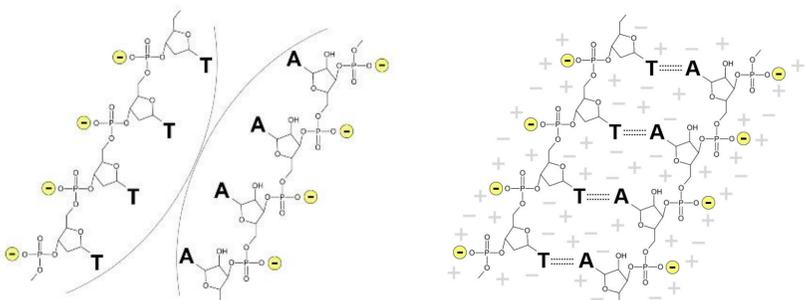


Figure 1: Schematic presentation of electrostatics repulsion between mRNA and column ligand, preventing hybridization between mRNA and Oligo dT

Figure 2: Schematic presentation of T-A base pairing between Oligo dT nucleotide and poly(A) tail of mRNA in high salt conditions.

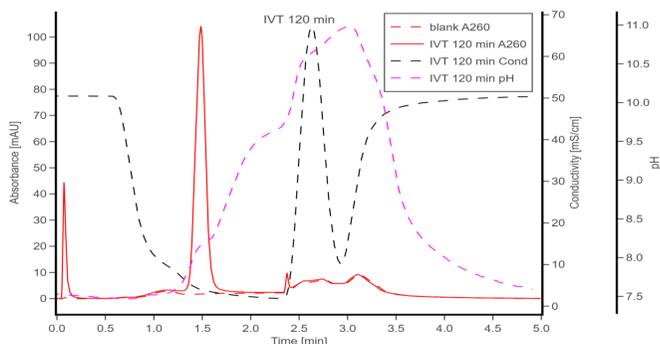


Figure 3: Representative analytical chromatogram for quantification of IVT mFix4 mRNA sample, using CIMac Oligo dT column, observing FT (flow through) species and poly(A) mFix4 mRNA.

2. Quantification of mFix4 mRNA

The developed HPLC method was validated using the mFix4 mRNA standard, following FDA and EMA guidelines. Using the calibration curve generated by PATfix software, developed in-house to support data processing and visualization, mRNA can be detected at a low limit of 25 ng.

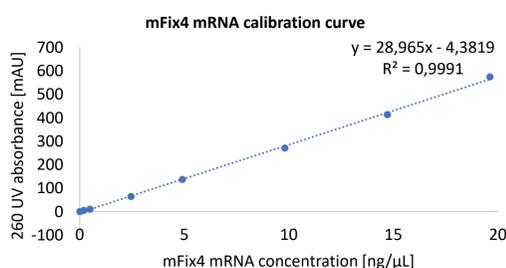


Figure 4: calibration curve of mFix4 mRNA using Oligo dT analytical method. Validated concentration range from 0.5 ng/μL to 20 ng/μL was tested with 0.9991 linearity.



Figure 5: PATfix® HPLC system.

3. mRNA quantification - IVT samples

Oligo dT analytical method enables at-line mRNA quantification during an mRNA transcription reaction process. The developed analytical method enables quantification of mRNA whilst detecting impurities eluted in the FT. Non-binding species in the flow through (FT) are expected to be nucleotides, DNA template and truncated mRNA (mRNA lacking poly(A) moiety). In the main peak, target mRNA is eluted.

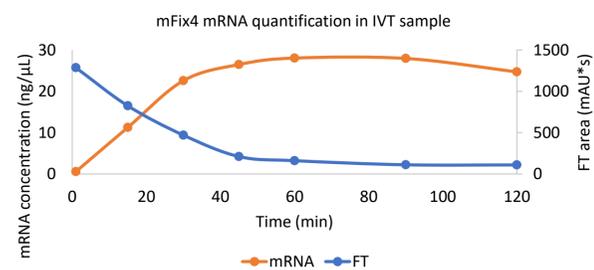


Figure 6: Fraction analysis of time course during IVT production to quantify mRNA. FT decreases with time, and mRNA concentration is increasing.

4. Process purification monitoring

Purification tracking can be performed using Oligo dT analytical method (Figure 6). An example of mRNA purification process using a CIMmultus™ Oligo dT column is presented below (Figure 7).

1) Pre-purification sample - Oligo dT Load.

Flow-through and elution peaks are observed.

IVT components eluting in FT and mRNA with poly(A) moiety eluting in the main peak.

2) Oligo dT flow through

Species, which do not interact with a CIMmultus column also do not interact with the CIMac Oligo dT column, as only an FT peak is observed, confirming sample composition.

3) Oligo dT wash sample

The wash step in the purification process is used to eliminate nonspecifically bound components from the column. As seen from the analytical run low concentrations of nonspecific contaminants are present.

4) Oligo dT Elution

Poly(A) mRNA product is produced. FT represents about 2% of the whole area of the chromatogram, presumably from RNA instability. Column selectivity was confirmed by reloading the FT fraction onto the analytical column (data not shown), where the fraction elutes once again in the FT.

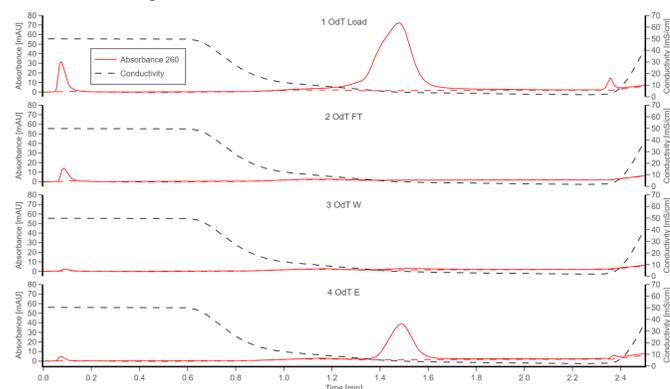


Figure 6: Fraction collected from CIMmultus Oligo dT chromatographic purification step and analysed using CIMac Oligo dT analytical column: 1) load Oligo dT sample - mixture from IVT reaction with IVT reaction contaminants 2) FT from prep run elutes also in flow through on analytical run 3) wash fraction - low concentration nonspecific bounded contaminants 4) Oligo dT elution - main product, pure mRNA with polyA tail.

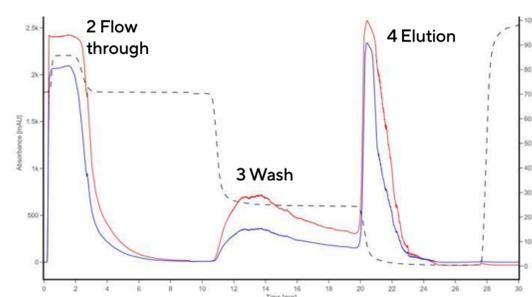


Figure 7: Prep chromatogram of mFix4 mRNA purification using CIMmultus Oligo dT column.

3. Conclusion

HPLC analytical method using CIMac™ Oligo dT column and PATfix software for data analysis is presented. Separation is based on A-T base pairing between dT oligonucleotide and poly(A) tail of the mRNA.

CIMac™ Oligo dT analytical method enables at-line mRNA quantification during an IVT reaction, separating polyadenylated mRNA from the transcription reaction process components.

Purification monitoring after one chromatographic step can also be performed using the CIMac™ Oligo dT analytical method.

4. References

Jalkanen, Aimes L. et al. "Determinants and implications of mRNA poly(A) tail size--does this protein make my tail look big?." Seminars in cell & developmental biology vol. 34 (2014): 24-32. doi:10.1016/j.semcdb.2014.05.018
Kowalski, P. S., Rudra, A., Miao, L., & Anderson, D. G. (2019). Delivering the messenger: advances in technologies for therapeutic mRNA delivery. Molecular Therapy, 27(4), 710-728.
FDA-2013-D-1020, EMEA/CHMP/EWP/192217/2009