Simplifying Progress

Chromatographic Platform for Characterization of mRNA Samples

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Introduction

mRNA has been at the forefront of both scientific and general public interests from the start of the COVID-19 pandemic. However, there are still limited options available for rapid characterization of mRNA containing samples. For precise characterization of an mRNA sample, first the presence and concentration of mRNA molecules in the sample needs to be identified. In the second step, any contaminants in the sample coming from the IVT reaction need to be identified and quantified. All major components of the IVT reaction; nucleotides, capping reagent, enzymes and DNA template may be present in the mRNA sample. In addition, impurities such as shorter, incomplete RNA fragments, and in particular, dsRNA may also be present. Contaminants may also come from the mRNA *in vitro* instability, caused by spontaneous hydrolyzation of the mRNA backbone. These issues can be mitigated using appropriate analytical tools throughout the mRNA production and purification steps.

1. PATfix™ mRNA Analytical Platform

PATfix™ analytical system with embedded, thoroughly validated methods provides a platform for robust mRNA characterization of complex samples using three complementary analytics:

- Oligo dT: affinity chromatography with CIMac™ Oligo dT column;
- PrimaS: bimodal anion exchange and hydrogen bonding with CIMac PrimaS™ column;
- SDVB: reverse phase with CIMac™ SDVB column.

Oligo dT: Affinity Chromatography With CIMac™ Oligo dT Column

The most important part of sample characterization is the mRNA quantification, which can be carried out with the Oligo dT analytics in a wide range of complex samples. This affinity column only binds the mRNAs that have a polyadenylated tail (polyA). They elute in the elution step. The species without a polyA tail elute within the first minute under non-binding conditions in the flow-through, as shown in Figure 1.

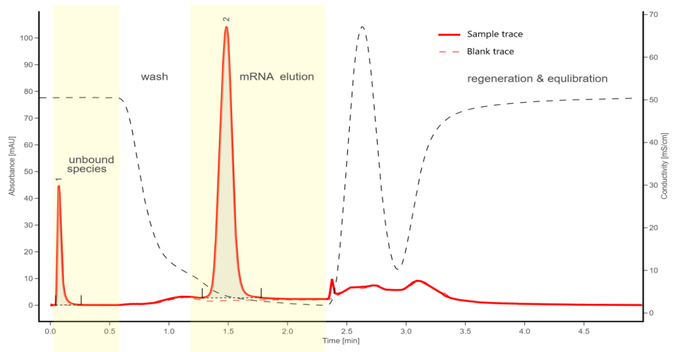


Figure 1: Characterization of an IVT reaction sample on a CIMac™ Oligo dT column (UV 260 nm).

Table 1: Oligo dT analytical method details

Mobile phase A	30 mM HEPES, 30 mM Na-phosphate, 0.5 M NaCl, pH 7.4
Mobile phase B	30 mM HEPES, 30 mM Na-phosphate, pH 7.4
Mobile phase C	10 mM CAPS, pH 11
Mobile phase D	0.5 M NaOH
Detection	UV 260 nm, conductivity, pH
Sample amount	0.5 µg
System	PATfix™ mRNA analytical system

PrimaS: Bimodal Analysis With CIMac PrimaS™ Column

Using CIMac PrimaS™ column, a full at-line analysis of the IVT reaction components can be achieved with a method able to quantify throughout the IVT reaction the depletion of individual nucleotides, capping reagent, and generation of mRNA, as shown in Figure 2.

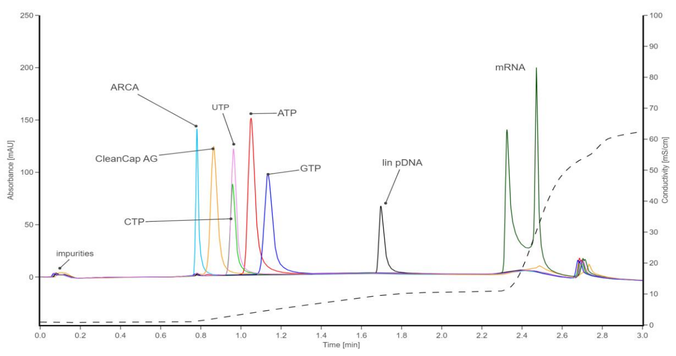


Figure 2: Elution profile of all major components of an IVT reaction analyzed on the CIMac PrimaS™ column (UV 260 nm). Full chromatogram and zoom-in to the area of interest.

Table 2: PrimaS analytical method details

Mobile phase A	50 mM HEPES, pH 7.0
Mobile phase B	50 mM HEPES, 100 mM $Na_4P_2O_7$, pH 8.3
Mobile phase C	100 mM NaOH, 1 M NaCl
Mobile phase D	0.5 M HEPES, pH 7.0
Detection	UV 260 nm & 280 nm, conductivity, pH
Sample amount	1 µg
System	PATfix™ mRNA analytical system

SDVB: Reverse Phase With CIMac™ SDVB Column

SDVB analytics provides data on mRNA integrity and size, and the amount of dsRNA in the sample.

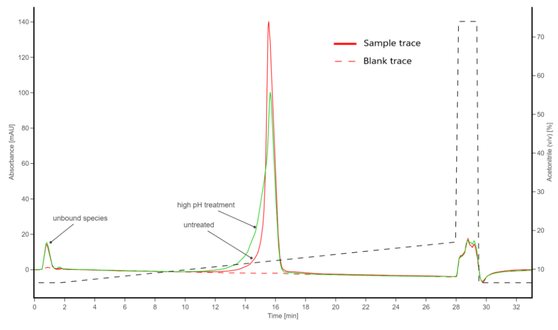


Figure 3: Example of untreated 4000 nt mRNA (red) and mRNA incubated at pH 11 (green) monitored at UV 260 nm.

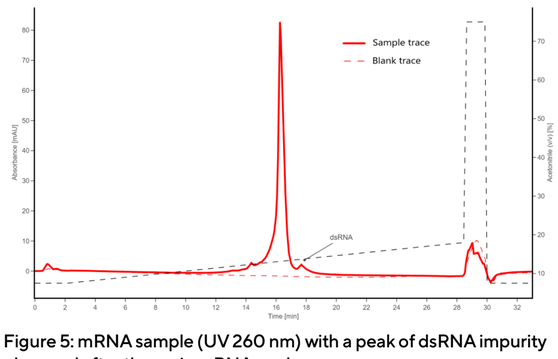


Figure 5: mRNA sample (UV 260 nm) with a peak of dsRNA impurity observed after the main mRNA peak.

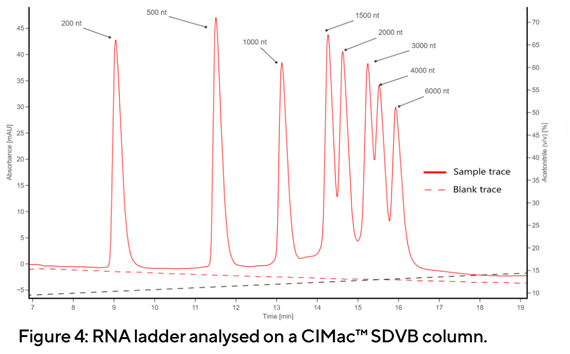


Figure 4: RNA ladder analysed on a CIMac™ SDVB column.

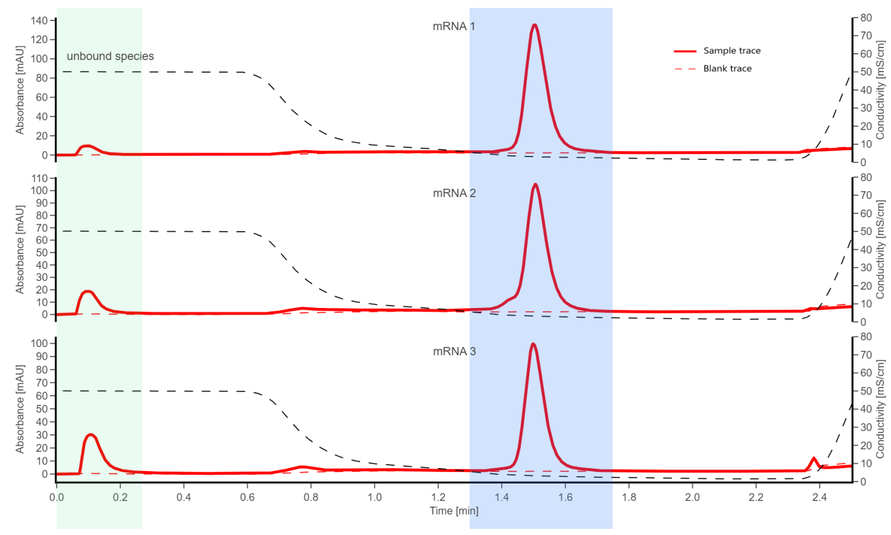
Table 3: SDVB analytical method details

Mobile phase A	50 mM TEAA, 7.5% Acetonitrile, pH 7.0
Mobile phase B	50 mM TEAA, 18% Acetonitrile, pH 7.0
Mobile phase C	50 mM TEAA, 75% Acetonitrile
Temperature	60°C
Detection:	UV 260 nm
Sample amount	0.5 µg
System	PATfix™ mRNA analytical system

2. Case Study: Characterization of Unknown mRNA Samples Using PATfix™ mRNA Analytical Platform

Determination of impurities in the final product, introduced during IVT reaction (such as DNA template, unincorporated nucleotides, mRNA fragments, double-stranded RNA) or purification process is a critical step in any development and manufacturing of drug substances. The developed selective analytical methods, which utilize three different column chemistries, deliver an analytical platform for mRNA quantification and characterization.

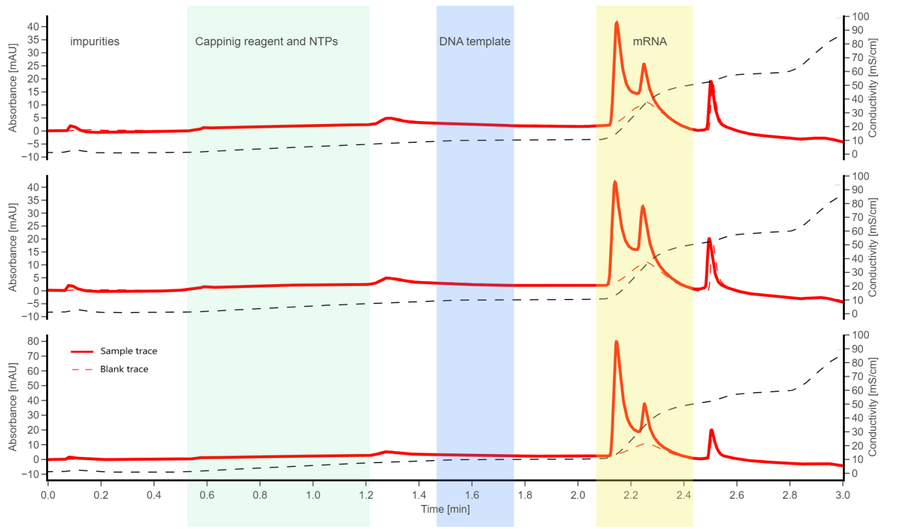
Oligo dT Analytics



Unbound species are observed in all three samples:
mRNA 1 6%
mRNA 2 13%
mRNA3 23%

Figure 6: Characterization of three unknown mRNA samples using CIMac™ Oligo dT column, UV260 nm. Unbound species - impurities can be seen in all three samples.

PrimaS Analytics



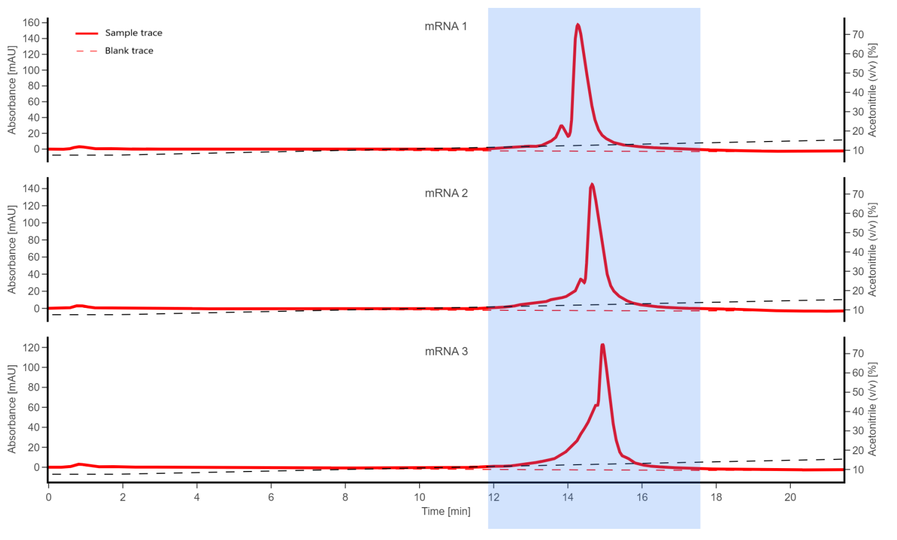
No peaks representing:
- Capping reagents
- Nucleotides (NTPs)
- DNA template

Only RNA peak is observed.

Unbounded species must represent shorter fragments of mRNA, or dsRNA.

Figure 7: Characterization of three unkown mRNA samples using CIMac PrimaS™ column. No peaks at the retention times of impurities from the IVT reaction can be seen. Full chromatogram and zoom-in to the area of interest.

SDVB Analytics



Fronting of the peak observed for all samples

In SDVB analytics, fronting represent shorter fragments of mRNA

No peak representing dsRNA is observed.

Impurities detected using Oligo dT are shorter RNA fragments.

Figure 8: Characterization of the three mRNA samples on a CIMac™ SDBV column, UV 260 nm. Fronting on the main mRNA peak is observed in all three samples.

3. Conclusion

In this work, PATfix™ mRNA analytical platform is presented. Platform combines three different chromatographic methods specially developed and validated for characterization of mRNA samples during upstream and downstream process. The versatility of the mRNA platform was demonstrated on case study example, where three different mRNA samples was evaluated.