

## Fast HPLC Analytics for IVT Reaction Characterization and Tracking Using CIMac PrimaS™ and PATfix®

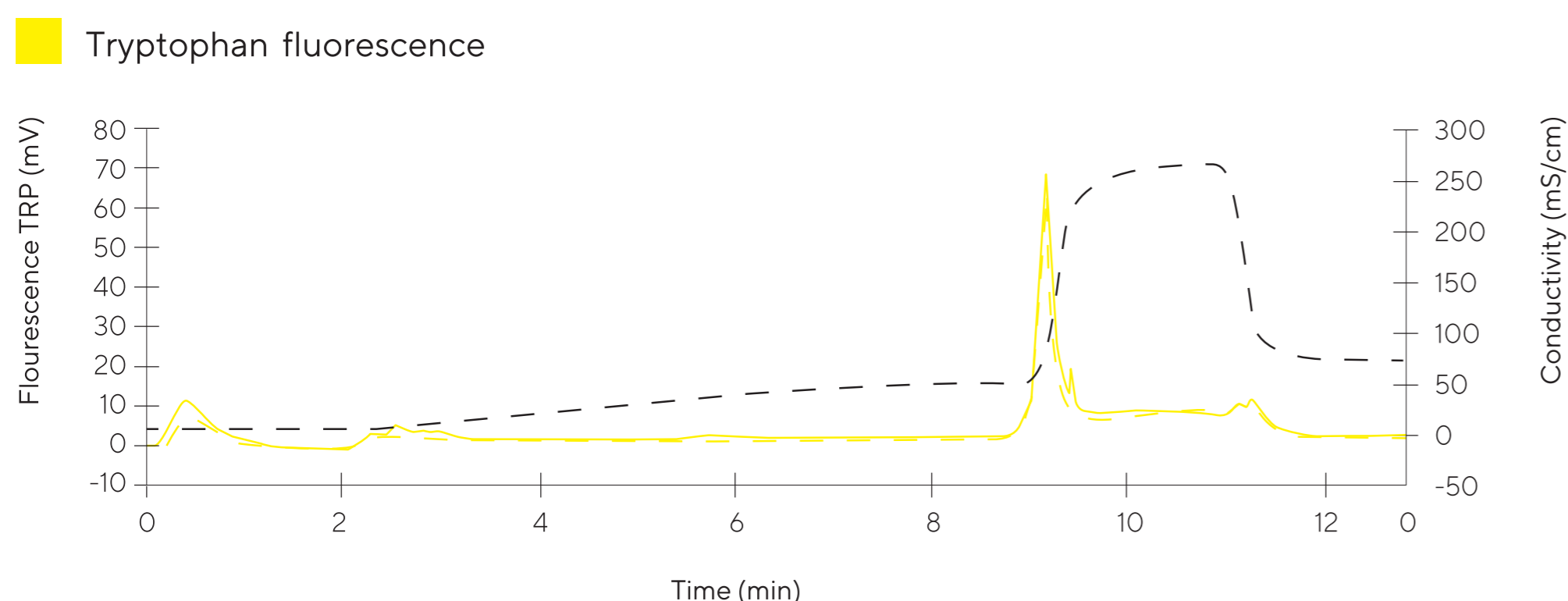
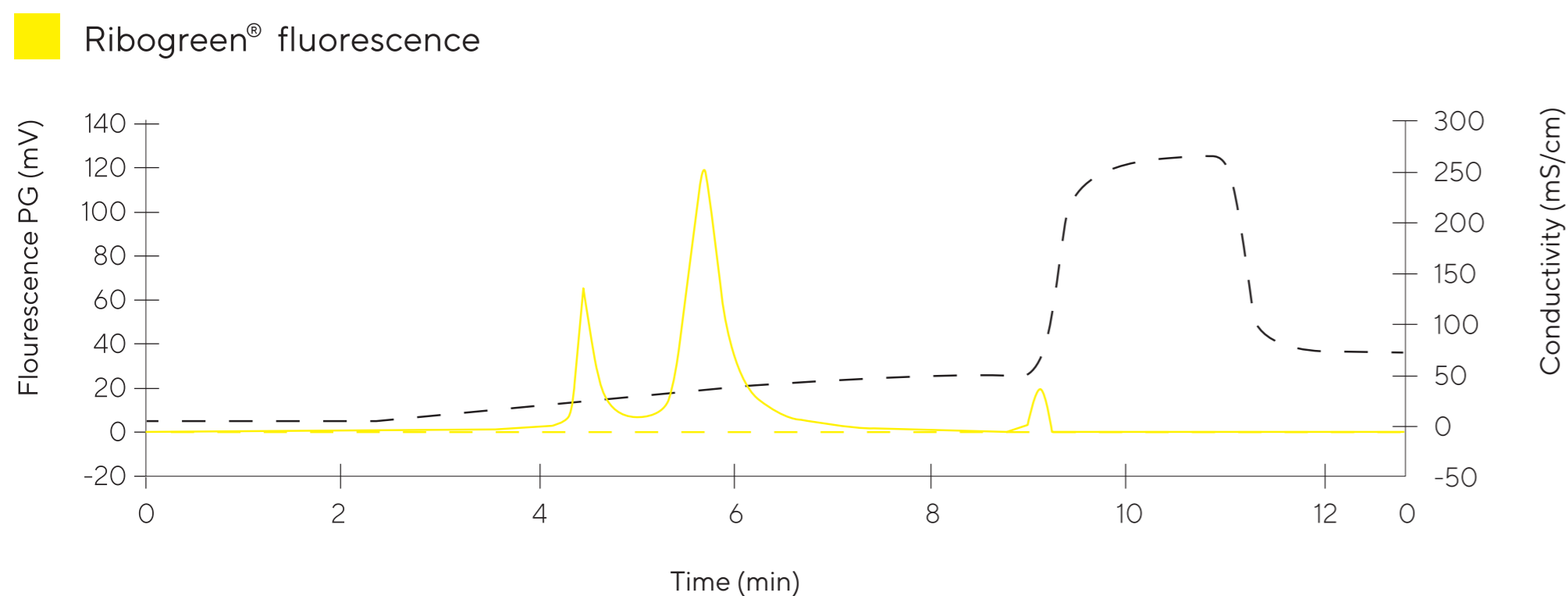
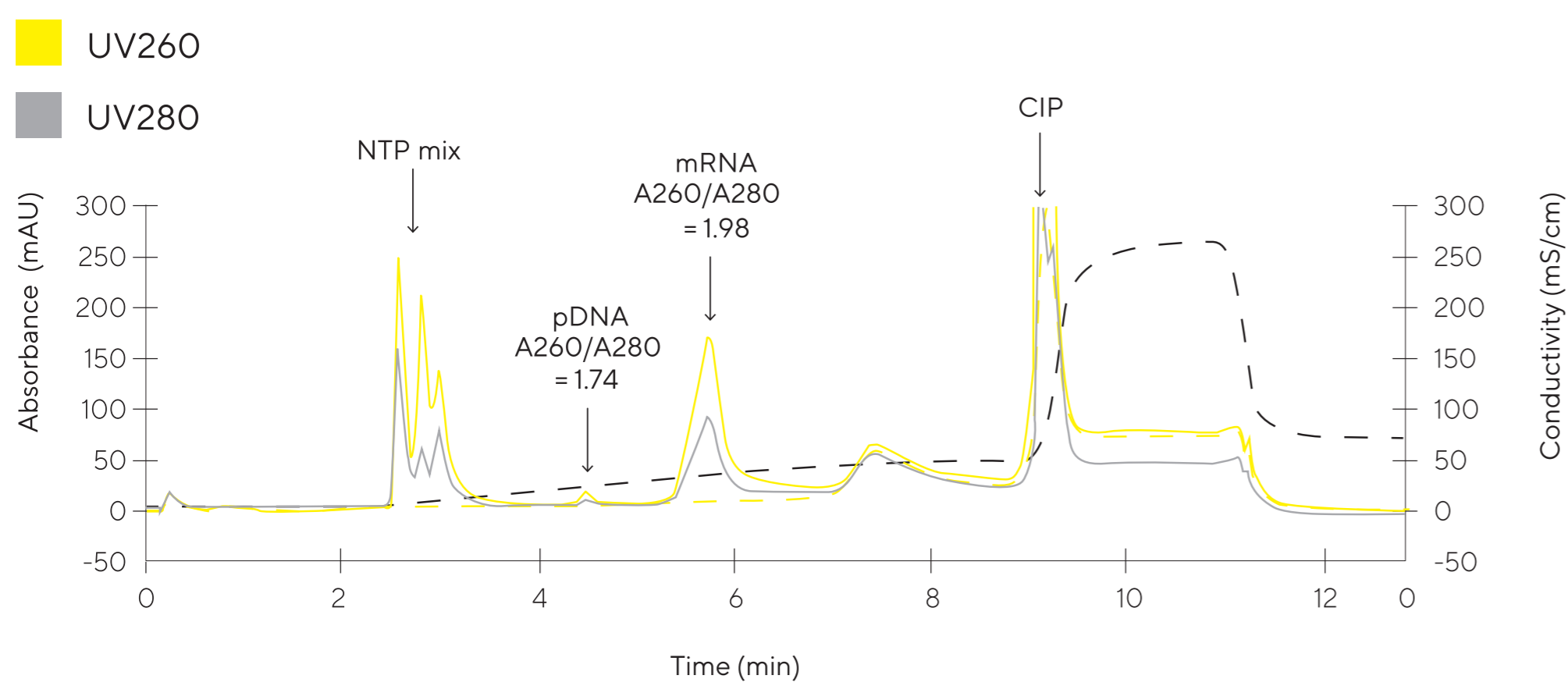
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### 1. Introduction

HPLC with convective chromatography media (e.g. monolith) offers a rapid analytical method to characterize complex biomolecular mixtures. Transcription reaction used for production of mRNA represents such a mixture, with biomolecular components varying in size, chemical and physical properties. A new PATfix® analytical HPLC approach presented here uses CIMac PrimaS™ to separate IVT components such as NTPs, capping reagent, enzymes, DNA template and mRNA in a very short gradient, opening the door for fast "at-line" tracking.

### 2. Sample Mixture Biomolecular Component Determination Using Multi-Detector PATfix®

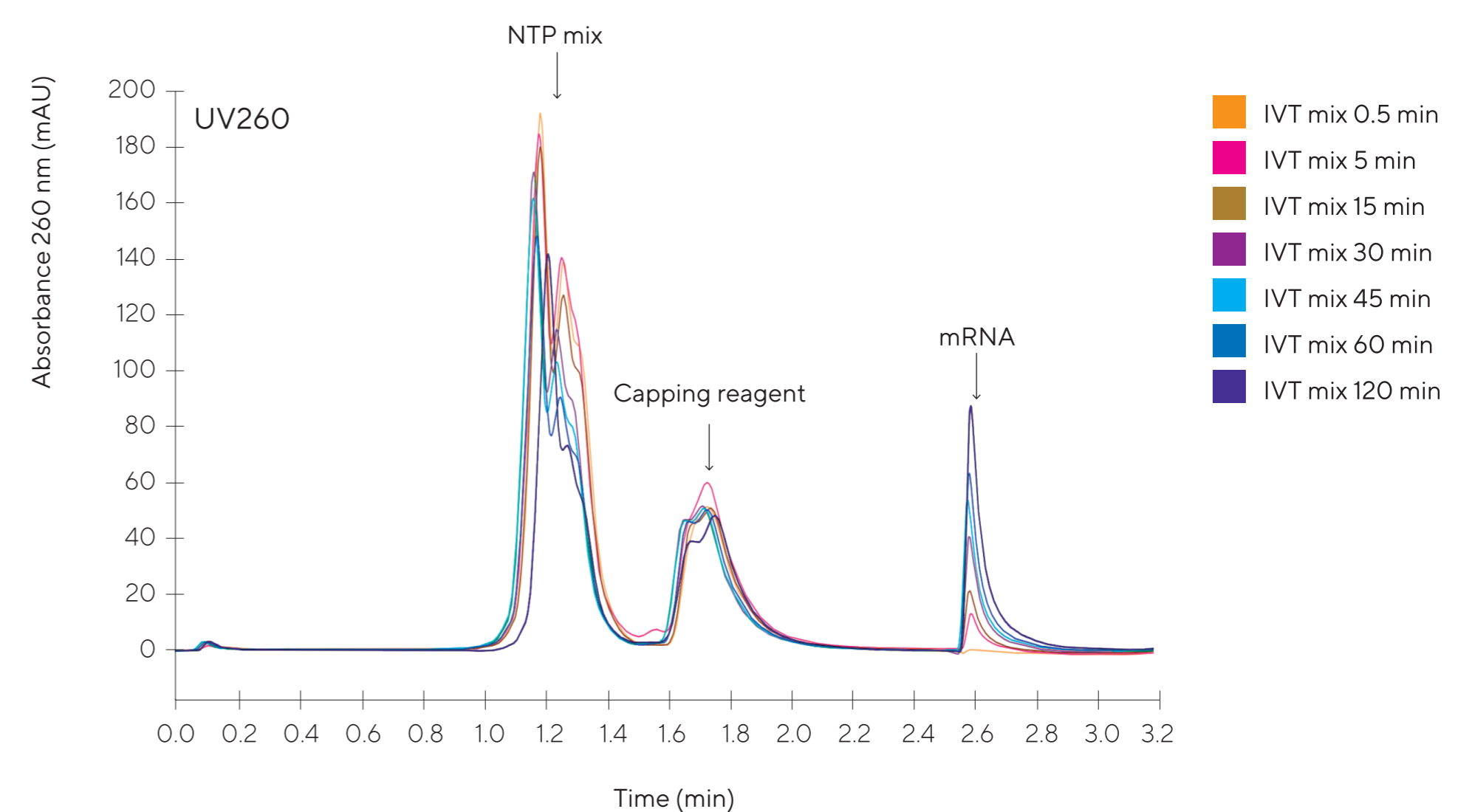


**Figure 1:** CIMac PrimaS™ 0.1 mL (Prod# 110.5118-2) on PATfix™ HPLC system, sample: IVT mixture incubated with Ribogreen® (ThermoFisher) and diluted in MPA, MPA (B): 50 mM MES pH 6.0 (+ 200 mM pyrophosphate) pH 6.0, MPC: 1 M NaOH + 2 M NaCl, MPD: 1 M CH<sub>3</sub>COONH<sub>4</sub>, Flow rate: 1 mL/min, method: 100 µL injection, 2 min MPA wash, 5 min gradient 0 to 100% MFB, 2 min hold, 2 min MPC, 4 min MPD, re-equilibration. Detection: absorbance (260 nm, 280 nm), tryptophan fluorescence, Ribogreen fluorescence.

PATfix® platform can be used to separate and characterize IVT mixture components. UV absorbance detects both protein and nucleic acid species. The ratio of absorbance at 260 nm and 280 nm can indicate whether a species is nucleic acid or protein. Tryptophan fluorescence allows identification of proteins and peptides. Nucleic acids do not contribute to the signal. Ribogreen® fluorescence is used to detect DNA and RNA species. Proteins and NTPs do not interact with Ribogreen® and do not contribute to the signal.

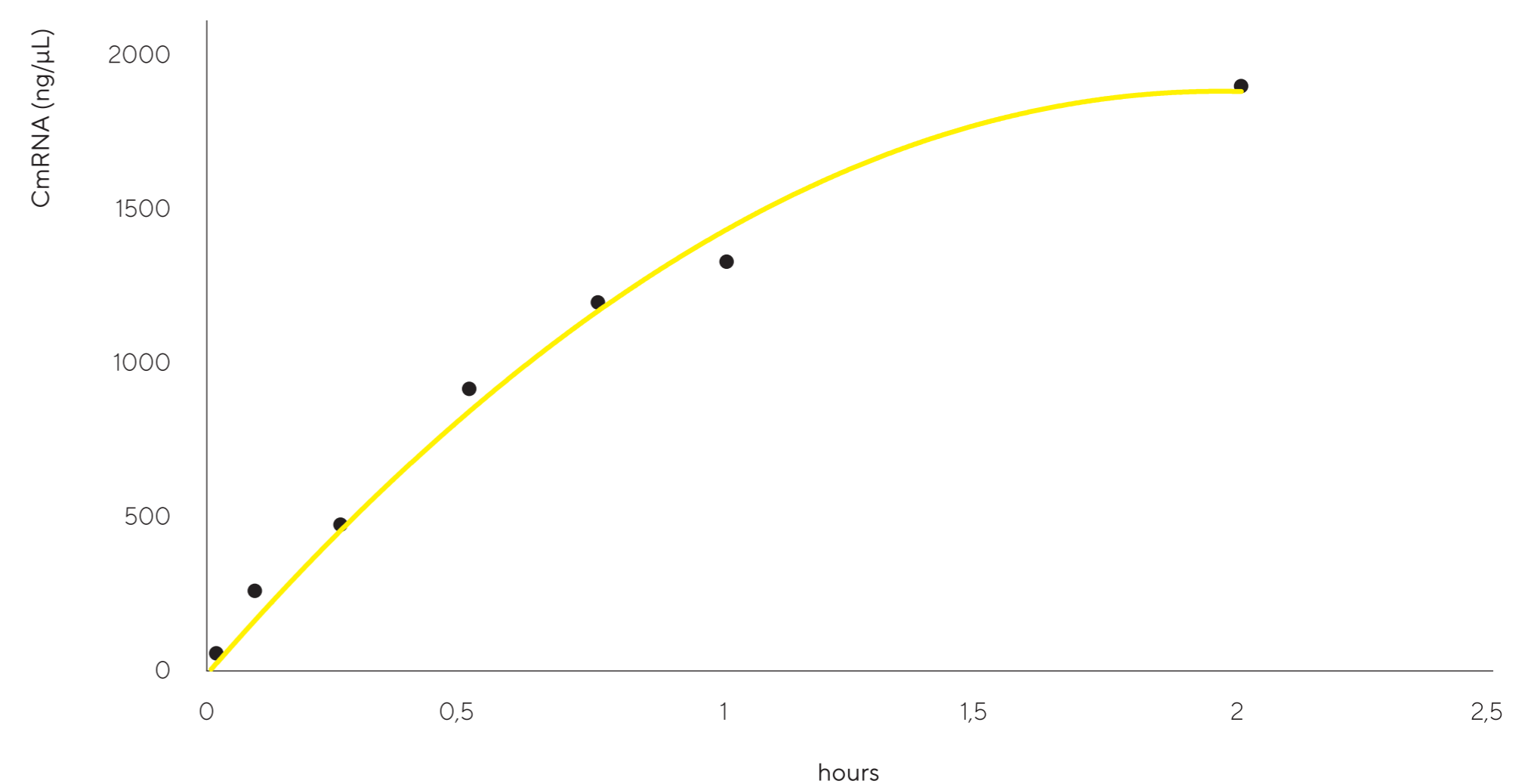
Using the above detection methods a clear determination of biomolecular species in the chromatogram can be made.

### 3. Tracking of IVT Reaction Kinetics by Rapid "At-Line" PATfix® HPLC mRNA Platform



Rapid, time optimized, analysis of IVT mixture at successive time points can be used to visualise progress of the IVT reaction using PATfix® software. The peaks at 1.2 min contain NTPs while the peak at 1.7 min, contains the capping reagent (CleanCap®, TriLink) and both can be seen decreasing due to consumption. The IVT-synthesized mRNA peak at 2.6 min increases with time. The peak area can be correlated with RNA concentration to assess reaction progression, allowing for rapid "at-line" tracking of the IVT reaction during production runs.

### 4. IVT Reaction Time Course



### 5. Conclusions

- CIMac PrimaS™ shows different selectivity for all of the IVT mixture components, through multiple binding mechanisms
- mRNA and DNA in IVT mixture are clearly separated
- Minimal sample preparation is required to analyse crude IVT samples allowing rapid "at-line" tracking
- PATfix® HPLC equipped with multiple detectors enables easy identification of different biomolecule species in one run
- Rapid HPLC analytical method can be used to follow and quantify IVT reaction kinetics "at-line"
- The presented technology can be used during process optimisation and for in-process control