Effect of Mg	extsuperscript{2+} Ion Concentration on IVT Reaction Kinetics Determined by Novel Rapid Analytical HPLC Assay

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Analytical Bottleneck in IVT Optimisation

The IVT reaction is one of the most expensive steps in mRNA production process and its optimisation to reach high mRNA yield is of key importance. Standard mRNA quantification techniques like absorbance and fluorescence based assays are time consuming and cannot be performed at line as the IVT reaction progresses. In addition, other reaction components like nucleotides and pDNA interfere in the analytical results and reduce the method’s accuracy. A new approach shown here uses CIMac PrimaS® analytical HPLC column to separate and quantify several key IVT components with a very short run time, enabling fast “at line” tracking.

Optimization of MgCl	extsubscript{2} Concentration in IVT Using PrimaS® Analytics

The concentration of MgCl	extsubscript{2} is one of the critical parameters in the IVT reaction. Seven identical reactions (20 µg/mL linear pDNA, 500 U RNA polymerase per µg pDNA, 4 mM ATP, CTP, UTP and GTP each, 1U/µL RNAse inhibitor, 1U/mL pyrophosphatase), varying only in the concentration of MgCl	extsubscript{2} (6 mM, 9 mM, 12 mM, 15 mM, 20 mM, 25 mM, and 50 mM) were analysed at different time points (0 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, and 4 h) on CIMac PrimaS® (N = 1 for each reaction). An overlay of chromatograms at all time points in Figure 3 shows consumption of nucleotides, and production of mRNA. Depletion of nucleotides can be observed after 1 h.

Conclusion

- A new analytical assay with CIMac PrimaS® shows different selectivity for all of the IVT mixture components, requires minimal sample preparation, and provides rapid quantitative results, “at line.”
- The method allows measurement of reaction kinetics and productivity in different conditions.
- Depletion of reagents (capping reagent, nucleotides) can be detected.
- The same method allows in process control during mRNA purification.
- The method was applied here to investigate the effect of magnesium ion concentration on mRNA production kinetics.