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CHROMATOGRAPHIC TOOLS FOR OPTIMIZATION OF IVT REACTION AND IMPROVING MRNA PURIFICATION PROCESS

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The recently demonstrated efficacy of mRNA-based Covid-19 vaccines has shown the promise of this therapeutic format, but also highlighted the need for higher efficiency of mRNA production to meet enormous needs for global vaccine supply.

Typical mRNA production process involves three key steps: 1) plasmid DNA (pDNA) production in supercoiled (sc) isoform, linearization and purification, 2) in-vitro transcription (IVT) reaction and 3) mRNA purification. Here we present a chromatographic toolbox for integrated mRNA production from pDNA to mRNA purification, including in-process analytics.

The pDNA purification approach presented here was designed to fit the specific requirements of mRNA vaccines. It integrates a linearisation step before polishing (removal of unwanted isoforms) of plasmid DNA. The polishing step after enzymatic linearisation, separates linear pDNA from enzyme and other unwanted products. Supporting in-process analytical tools are presented.

IVT reaction monitoring with novel HPLC approaches includes CIMac PrimaS analysis of mRNA content as a function of time, with concomitant monitoring of NTP consumption. With information on NTPs, capping reagent and mRNA content, IVT reaction can be rapidly optimized for maximum productivity, in near real-time. Advantage of at-line monitoring is to prevent degradation of mRNA in IVT mixture which would occur after maximum productivity is reached.

Purification of mRNA from IVT reaction mixture can be achieved using selective binding to polyA tail (using OligodT chromatography) or multimodal chromatography (PrimaS) which separates the ssRNA product from DNA template and IVT reaction mixture. Polishing approaches for final separation of ssRNA and dsRNA using hydrophobic interaction chromatography (HIC) and reverse-phase (RP) chromatography achieve high purity of final product. Supporting in-process analytical HPLC tools, including multimodal chromatography resin, facilitate a rapid read-out of mRNA concentration and purity profile.