BioReliance®
Contract Testing Services

Technical Note

Determining RNA integrity and purity by Capillary Gel Electrophoresis

Analytical Development for mRNA

Challenge

RNA is inherently unstable and so it is susceptible to degradation during manufacture and storage. To ensure the quality of mRNA products, it is important to confirm the integrity of the RNA in terms of its size and length. A high-resolution analytical method is required to separate and quantify the full-length mRNA relative to any fragments that may be present in the sample.

Methodology

Capillary Gel Electrophoresis (CGE) is commonly employed for the analysis of RNA. Compared to traditional gel-based electrophoresis, CGE offers higher resolution separation, greater reproducibility, and improved quantitation. It also benefits from low sample consumption.

In CGE, a capillary is filled with a separation gel matrix containing a fluorescent dye. The RNA is injected, where it binds the fluorescent dye as it migrates through the capillary. RNA molecules are separated on the basis of size, with smaller fragments exhibiting a shorter migration time than larger molecules. The size of the RNA is estimated by comparison to an RNA reference ladder, which contains a mixture of different RNAs of known size.

Assay Details

A minimum of 5 µL mRNA at 25 µg/mL is required. The sample is diluted to the required concentration prior to analysis.

Case Study Results

To challenge the performance of the CGE method, a 2.5 kb mRNA was spiked into a 5.0 kb mRNA sample to simulate the presence of a smaller impurity in the final product. Solutions containing varying levels of the spiked 'fragment' were analyzed by CGE.

As shown in **Figure 1**, the smaller fragment is well resolved from the full-length mRNA. Relative to the RNA size ladder, the migration times of each peak are consistent with the expected sizes of the fragment and the intact mRNA.

Figure 2 shows the relative percentage area of the fragment peak compared to the theoretical (spiked) concentration of fragment. The method demonstrates good linearity, accuracy and precision over the range studied, with a limit of quantitation below 1%.

Accordingly, CGE provides a suitable method for the assessment of mRNA integrity and purity. Given that full-length mRNA is essential to the potency of the drug substance, this assay forms a critical part of lot release and stability testing.

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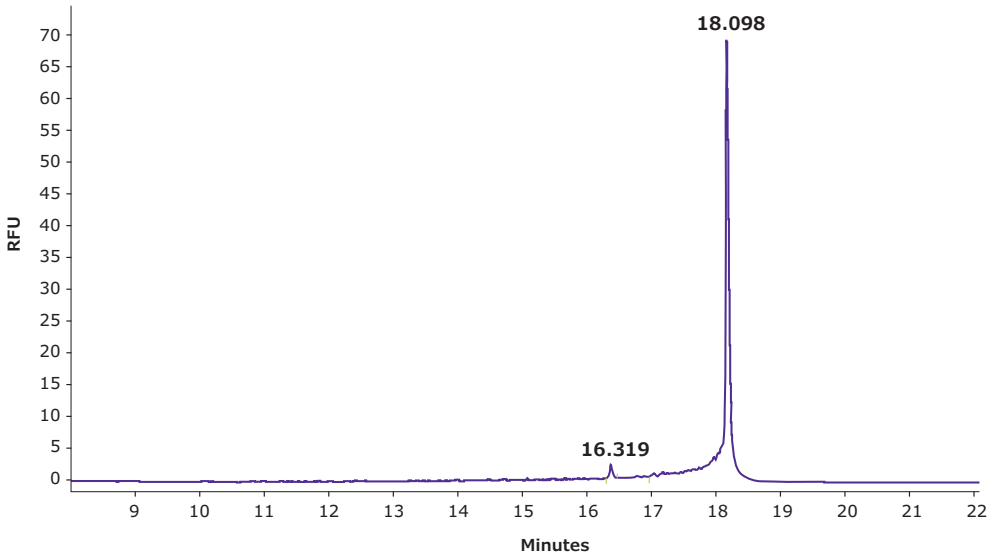

Figure 1: Electropherogram, showing resolution of 2.5 kb 'fragment' in 5.0 kb mRNA

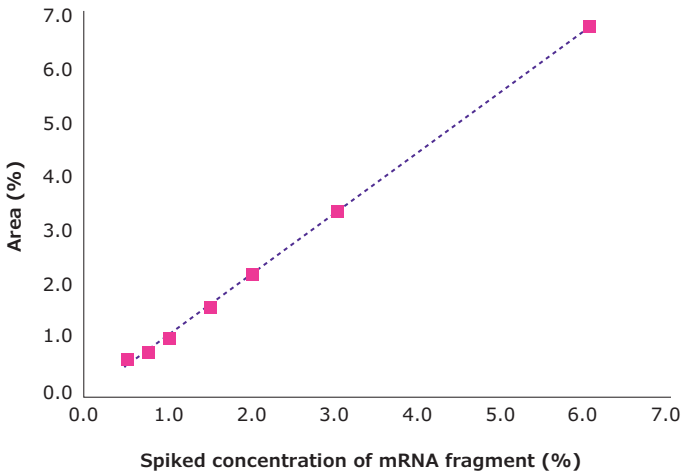

Figure 2: Observed percentage area for different levels of mRNA fragments

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