CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

mRNA fragmentation and quality assessment using ion pair reverse-phase analytics

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Rapid advancement of mRNA technology, as a response to the COVID-19 crisis, prompted an increased need for precise analytical methods to support the fast-paced mRNA process development. Accurate and robust analytics are required to support modifications in the mRNA production process, protocols, raw materials, *in vitro* transcription reaction, purification methods, scale-up, or final formulation processes, to ensure high quality and safety of the final product. This Innovator Insight demonstrates the application of an ion pair reverse phase chromatographic analytical method as a robust analytical tool to determine mRNA fragmentation while also separating *in vitro* transcription components from the main product. The method's efficacy is assessed through a comprehensive stability study of a mRNA standard at different temperatures. The chromatographic analytical results are compared to the ones obtained by the capillary gel electrophoresis, a well-established method for the analysis of fragmented mRNA.

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CHALLENGES OF mRNA FRAGMENTATION MONITORING

mRNA technology is a relatively new alternative to conventional vaccines showing great potential for infectious disease control and gene therapy. The highly effective mRNA-based vaccines enabled curbing of the COVID-19 pandemic and paved the way for the development of a broader range of innovative vaccines and therapies. The rapid pace of development and manufacturing of vaccines is one of the many advantages of the mRNA modality over other platforms, establishing the mRNA technology as a promising tool not only for addressing future pandemics, but

also for combating other infectious diseases like rabies, Zika, and cytomegalovirus infections. Furthermore, numerous mRNA-based therapies are currently progressing through clinical trial pipelines for the treatment of a wide range of diseases, including cystic fibrosis and various cancers [1].

Innovative products, such as mRNA vaccines must exhibit sufficient quality, safety, and efficacy. As modifications are introduced to raw materials, processing steps, and formulation during process development and the scale-up process, the implementation of rigorous analytics becomes essential to ensure the quality and safety of the final product. Inadequately identifying and addressing quality issues can jeopardize the integrity of the product, resulting in unfavorable clinical outcomes, costly delays, and potential challenges in obtaining regulatory approval. Therefore, it is crucial to proactively identify and mitigate any quality concerns to ensure the safety, efficacy, and timely delivery of mRNA-based products.

Ensuring the safety and reliability of a drug substance becomes significantly more manageable within a tightly controlled production and purification environment. To achieve this goal, rigorous QC steps must be executed at every stage of the process, demanding the utilization of robust and accurate analytical methods [2].

To address the need for mRNA characterization methods, regulatory agencies such as the US Pharmacopeia and US FDA are developing a set of analytical methods for mRNA quality. Their goal is to create a shared understanding of mRNA quality attributes with the aim of accelerating product development, guiding successful scale-up of manufacturing, and ensuring best practices and appropriate quality controls for this new modality. US Pharmacopeia guidelines for mRNA vaccine quality suggest ion pair reverse-phase high-performance liquid chromatography (IP-RP-HPLC) as a preferred analytical method for mRNA product-related impurities such as fragmented mRNA [2].

The developed chromatographic method enables detection of *in vitro* transcription (IVT) components while assessing mRNA fragmentation thus accelerating analytics of complex samples.

In this study, the suitability of the CIMac SDVB (styrene-divinylbenzene) chromatographic analytical method for the determination of the extent of mRNA fragmentation using a PATfix analytical chromatographic system was investigated [3]. The results from the SDVB analytics were compared to the data obtained by the widely accepted capillary gel electrophoresis (CGE) method.

VERSATILE mRNA QUALITY CONTROL METHOD

The CIMac SDVB column enables size separation of RNAs alongside the detection of impurities such as DNA template, nucleotides, and capping reagent (Figure 1). The method is also applicable for double-stranded RNA (dsRNA) impurity assessment (Figure 2).

Analysis of the complex IVT sample using the SDVB analytical method is presented in Figure 1. The analytical method enables separation of mRNA from IVT impurities, e.g., DNA template and dsRNA, while NTPs and capping reagents do not bind to the column. They elute in the non-bound peak and cannot be separated. The same analytical method is used for overall mRNA yield determination and estimation of fragmented mRNA.

One of the main impurities in the mRNA production process is dsRNA. The SDVB analytical method allows for the detection of dsRNA species due to its greater hydrophobicity relative to ssRNA, resulting in a longer retention time (Figure 2).

Size separation of RNAs is achieved due to differences in hydrophobicity, where the retention time is correlated with the length of the RNA molecule. Shorter fragments elute before the parent mRNA, making it suitable for mRNA quality assessment (Figure 3).

The separation of RNA molecules by size using SDVB analytics is presented in Figure 3.

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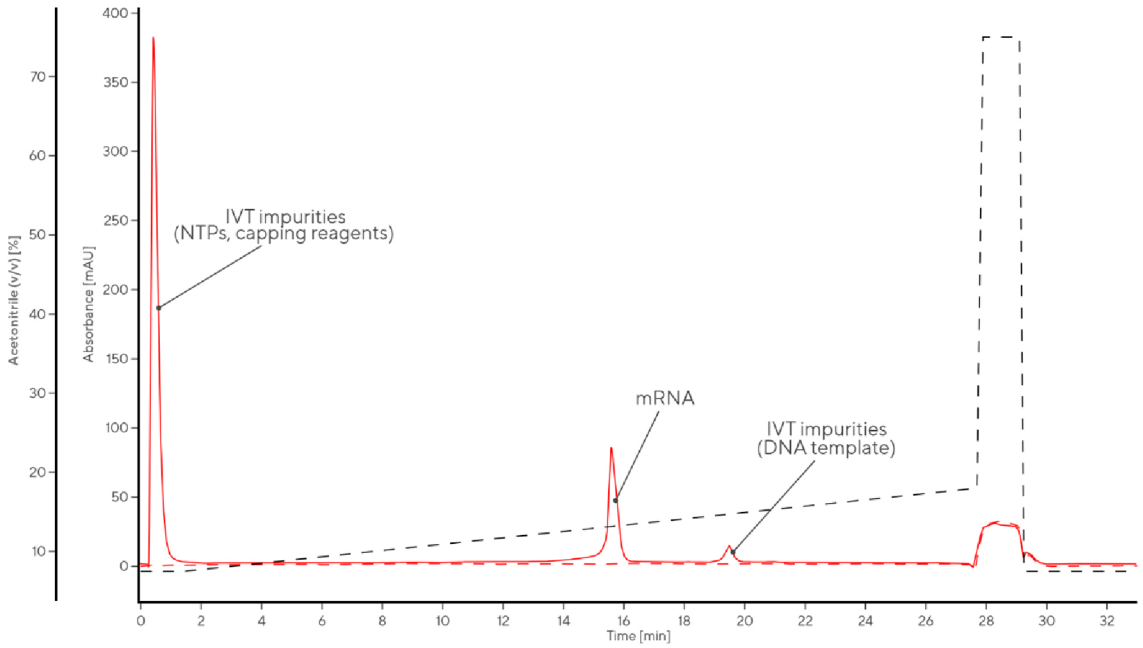
FIGURE 1

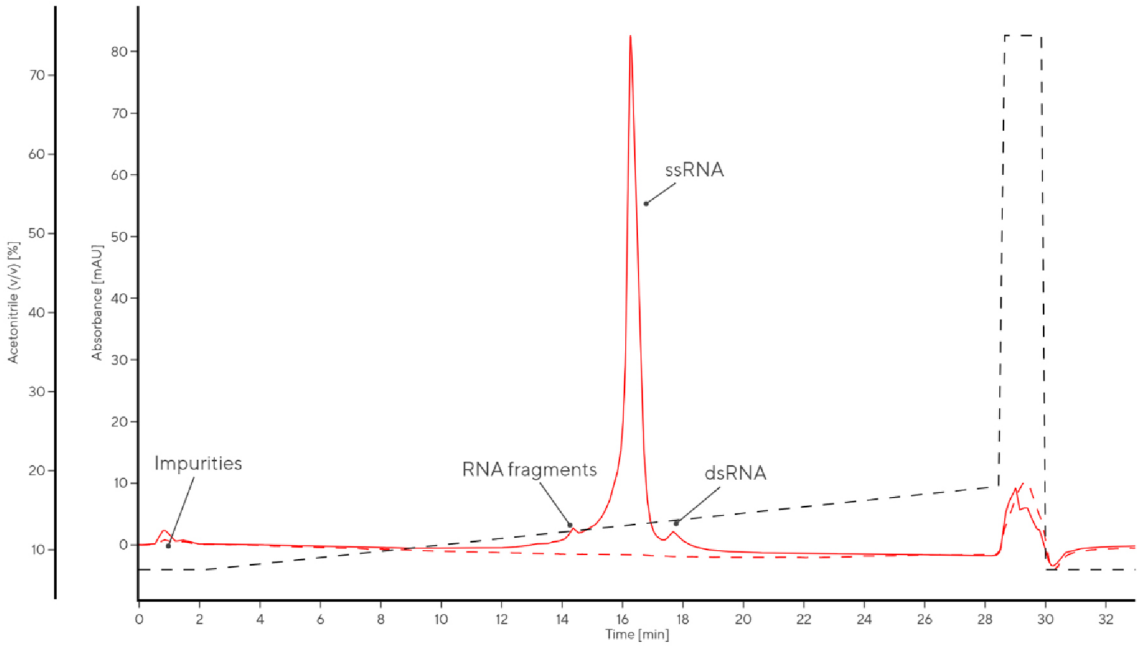
SDVB analysis of IVT sample.



The analytical method enables separation of IVT impurities from mRNA (red trace: UV 260 nm, black trace: acetonitrile gradient).

FIGURE 2

SDVB analysis of an mRNA (4000 nucleotides) with dsRNA impurity detected



Red trace: UV 260 nm; black trace: acetonitrile gradient.

RNA fragments, shorter than 50 nucleotides (nt) elute in the non-bound peak, while baseline separation of RNA fragments in sizes from 50–1000 nt is achieved. The robustness and reproducibility of the method are confirmed using a multiple ladders approach, where the fragments of the same size elute at the same retention time, regardless of the ladder provider.

CGE ANALYSIS OF IVT SAMPLE

RNA fragments are separated under denaturing conditions by size on a bare fused silica capillary filled with separation gel containing urea and polyvinylpyrrolidone. The gel is stained with Sybr® Green II dye, and mRNA fragments are detected using a LIF (laser-induced fluorescence) detector [4,5].

Capillary gel electrophoresis is used for monitoring fragmented RNA; however, impurities such as NTPs and capping reagents cannot be detected when the gel is stained with an intercalating fluorescent dye (Sybr® Green II dye). Another impurity that cannot be detected is dsRNA, due to denaturing conditions. The migration time of linear (lin) pDNA is shorter than the main mRNA peak and overlaps with RNA fragments and therefore cannot be identified. An example of the CGE analysis of an IVT sample, overlaid with lin pDNA, is presented in Figure 4.

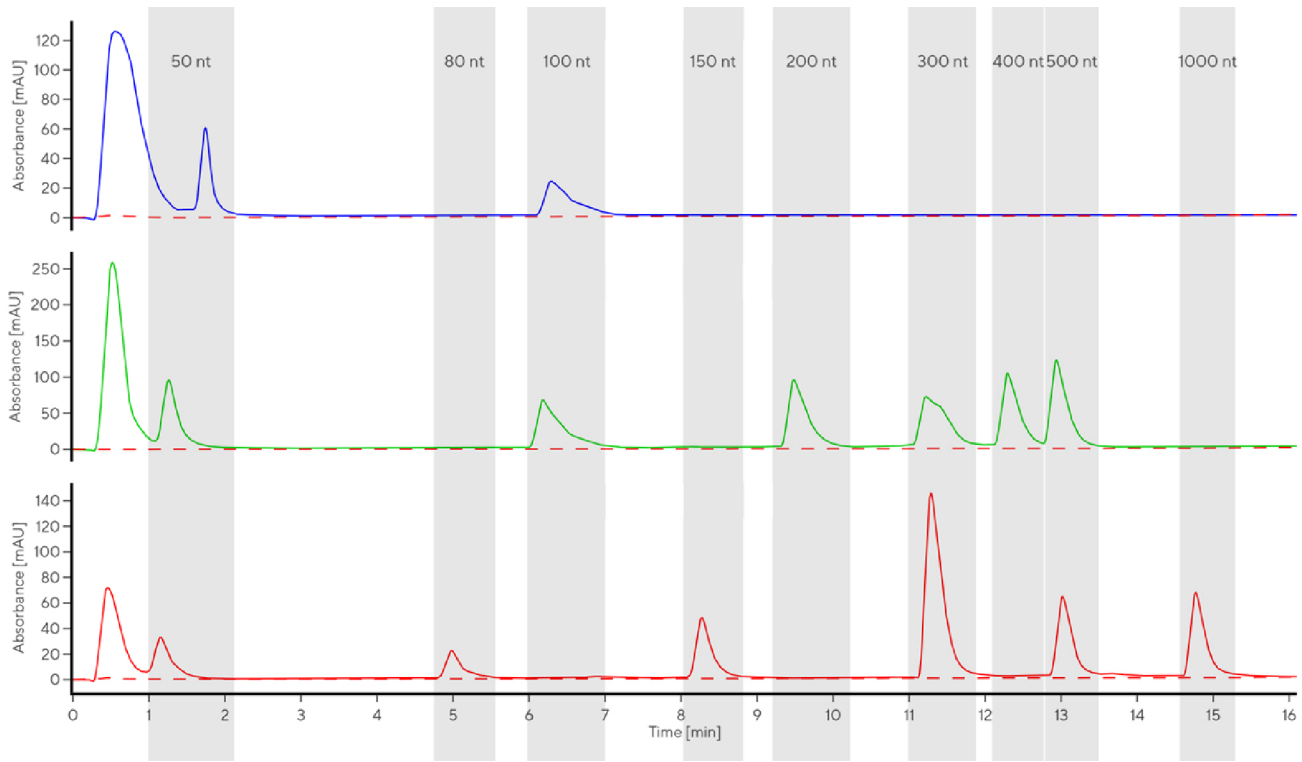
MATERIALS & METHODS

Experiments were performed using mFix4 mRNA analogue standard, a 4000 nt long uncapped mRNA with polyA tail (Sartorius BIA Separations product, Cat. No. BIA-mFix4.1.1).

Chromatographic analysis was performed using PATfix® analytical system (Figure 5) with a quaternary pump, a multiwavelength UV-VIS detector, a column thermostat, and a

FIGURE 3

Overlay of three commercially available small RNA Ladders using SDVB analytics (UV 260 nm).



Blue: Small RNA marker (Abnova); green: RNA marker low (Abnova); red: low range ssRNA ladder (New England Biolabs).

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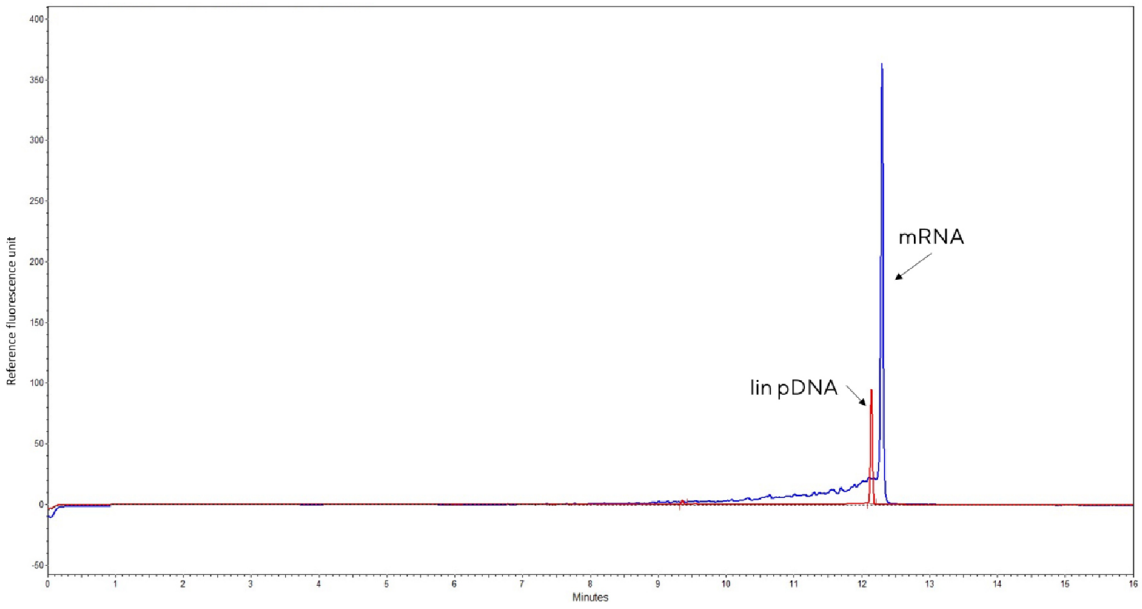
FIGURE 4

CGE analysis of an IVT sample (blue trace) overlaid with the lin pDNA sample (red trace).



mobile phase preheater. PATfix software was used for system control and data analysis.

The mRNA sample incubation was performed using a Thermo-Shaker from BioSan at an elevated temperature. The sample was diluted with mobile phase A prior to injection on the CIMac SDVB reverse phase monolithic chromatographic column from Sartorius BIA Separations (Table 1).

CGE analytics were performed using AB Sciex PA 800 Plus system with LIF detection (Table 2). The sample preparation consisted of a dilution to a target concentration of 1 µg/mL, heating the sample to 65 °C for 1 min followed by rapid cooling on ice and short centrifugation. The sample was injected electrokinetically.

EVALUATION OF FRAGMENTED mRNA

Data analysis to evaluate the extent of mRNA fragmentation, such as peak fronting of the main mRNA peak, was carried out by the PATfix embedded software. The PATfix algorithm determines peak fronting by evaluating the first derivatives (Df) of the absorbance signal (Figure 6), determining the maximum slope of the tangent to the chromatographic response [6,7], which defines the right-hand side border of the peak fronting area. The peak fronting area is proportional to the content of shorter RNA fragments, making it a valuable tool for fragmentation studies. The data analysis was further improved by applying a Savitzky-Golay numerical filter to smoothen the original signal. The PATfix algorithm ensures a robust and reproducible signal integration, independent of the analyst.

FIGURE 5

PATfix® chromatographic system

TABLE 1

SDVB analytical method details and gradient.

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, 7.5% acetonitrile
Temperature	60 °C
Detection	UV 260 nm
Injection amount	0.5 µg
Column	CIMac SDVB (0.3 mL, 2 µm channels)
System	PATfix® mRNA chromatographic system

SDVB: Styrene-divinylbenzene; TEAA: Triethylamine acetate.

TABLE 2

CGE method details.

Capillary	50 µm bare fused silica, total length 30 cm
Detection	LIF detector (Ex. 488 nm, Em. 520 nm)
Gel	PVP, Urea, Sybr® Green II
Injection	Electrokinetic
Separation	6 kV
System	PA 800 Plus

LIF: Laser induced fluorescence; PVP: Polyvinylpyrrolidone.

Determination of mRNA fragmentation by CGE was performed by peak integration. The red horizontal line represents the baseline while the vertical line represents a dropped line that separates the main peak from fragments as shown in Figure 7.

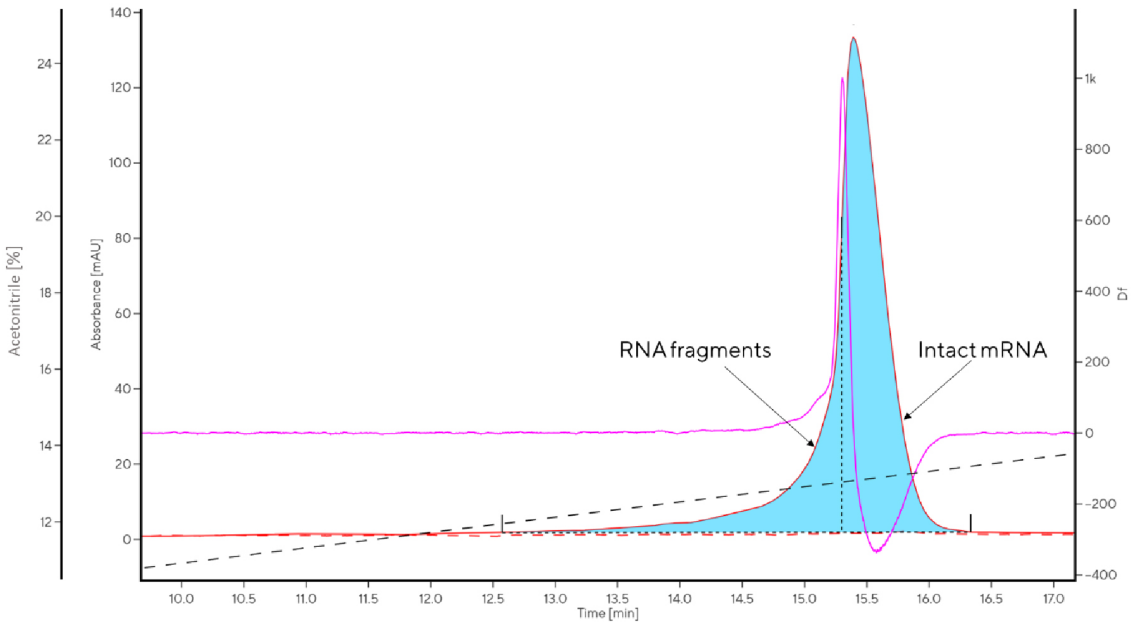
ASSESSMENT OF FRAGMENTED & INTACT mRNA

To demonstrate the method's ability to separate between fragmented and intact RNAs, fractions from an SDVB analytical run of the mRNA sample incubated at 60 °C for 6 h were collected and analyzed by the two analytics. The fractions were collected at the peak split determined by the Df, as shown in Figure 8. The chromatogram of the initial sample and the collected fractions analyzed with the SDVB method is presented in Figure 9.

The chromatogram of the initial sample and the collected fractions analyzed by the CGE method is presented in Figure 10.

FIGURE 6

Determination of fragment mRNA peak with the first derivative of the absorbance signal.



Red trace: UV 260 nm; pink trace: $Df_{UV\ 260\ nm}$; black trace: acetonitrile gradient.

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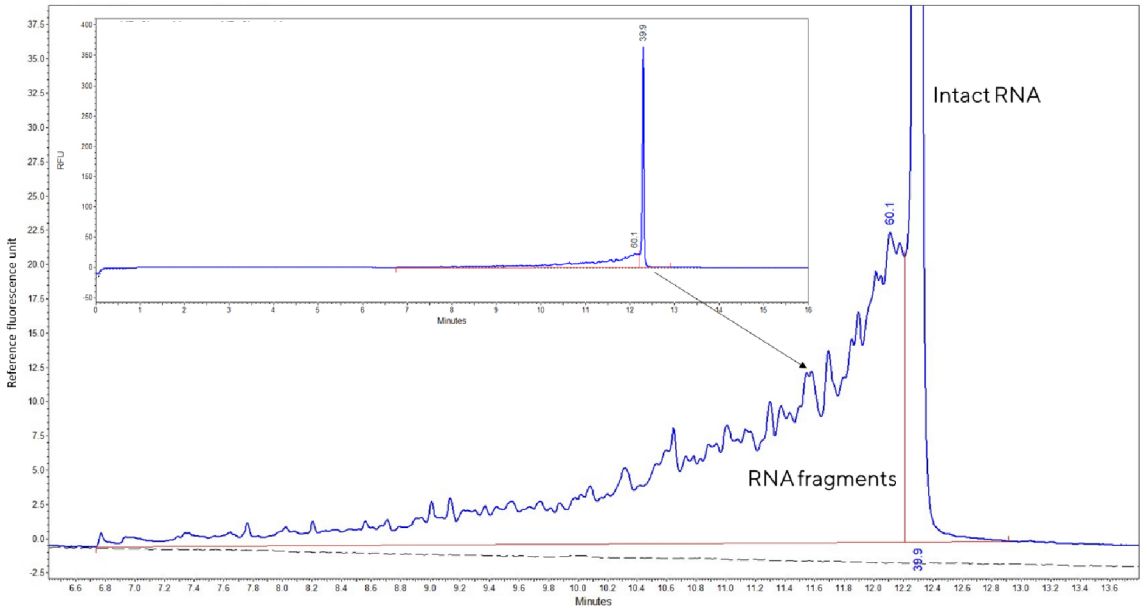
FIGURE 7

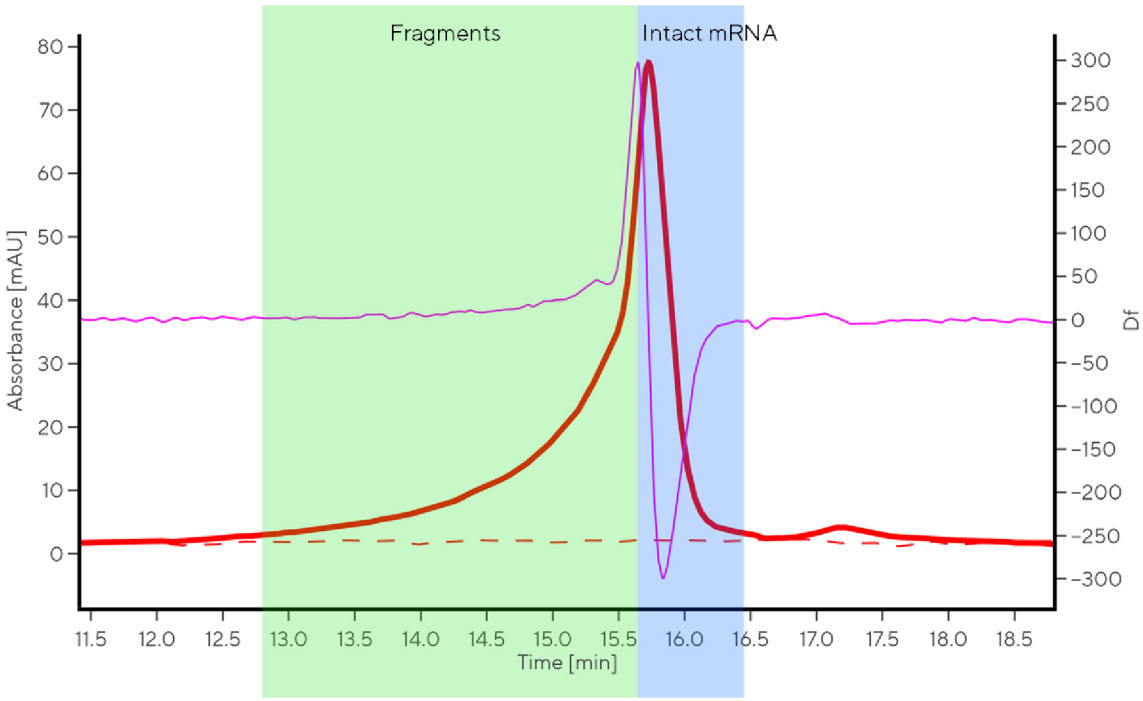
Determination of mRNA fragmentation using CGE analysis.



Blue trace: IVT sample; Black trace: blank sample.

FIGURE 8

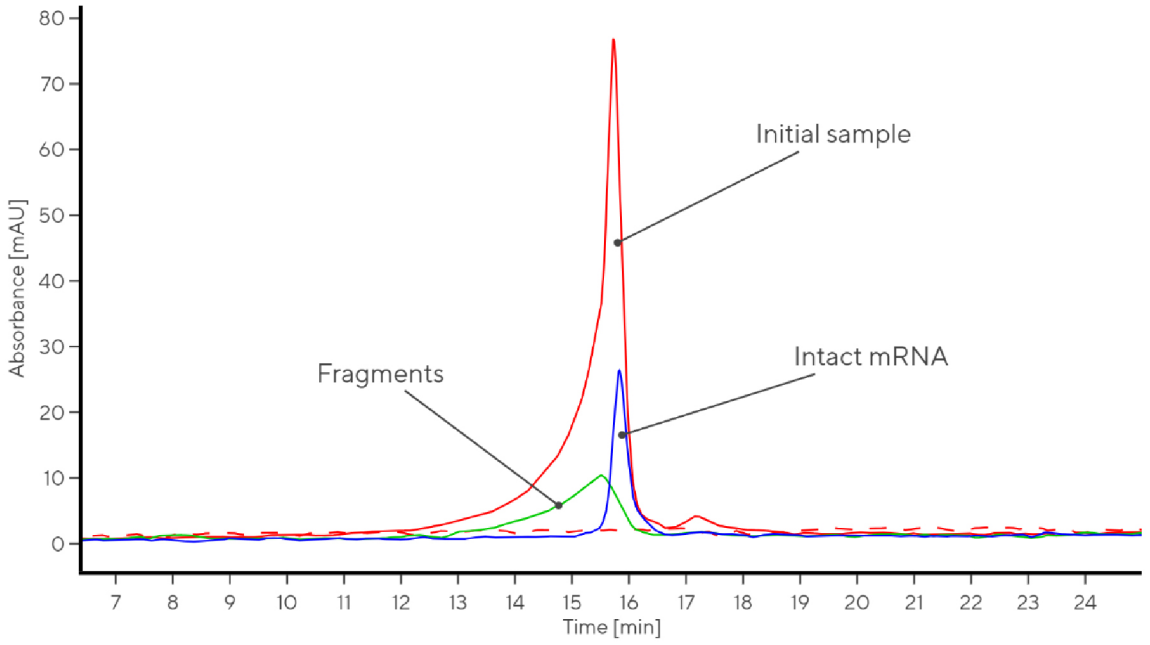
Fraction collection during the SDVB analytical run.



Fragments: green band; mRNA: Blue band; UV 260 nm: red trace; $Df_{UV\ 260\ nm}$: pink trace.

FIGURE 9

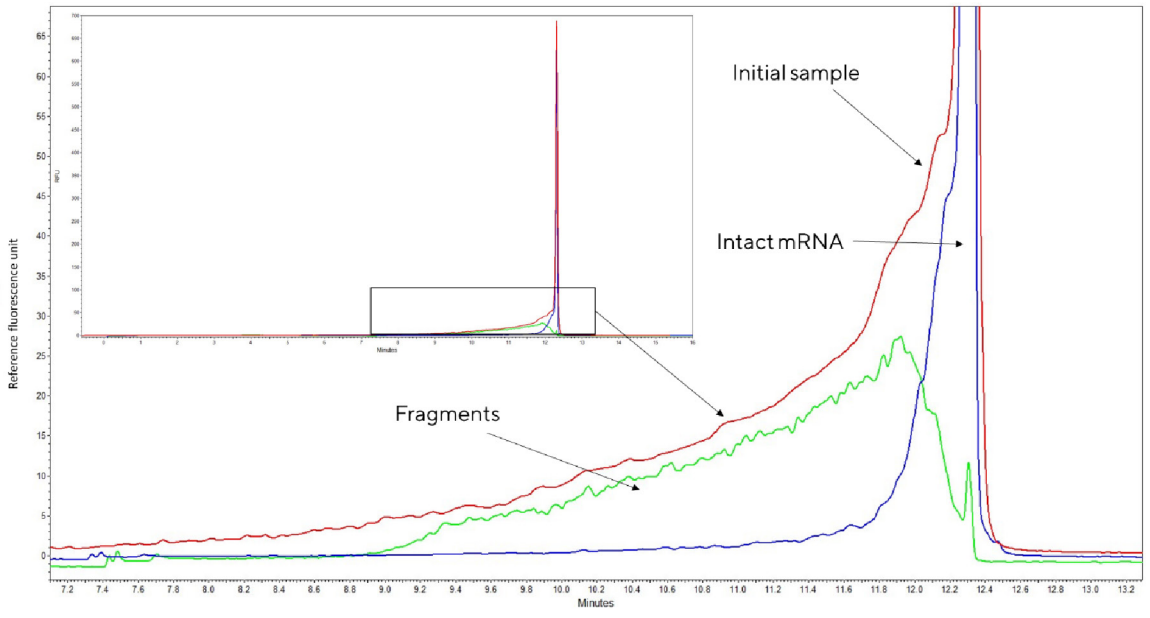
Separation of intact mRNA (blue trace) and fragmented mRNA (green trace) fractions on SDVB column.



Initial sample is presented with red trace (UV 260 nm).

FIGURE 10

Separation of intact mRNA (blue trace) and fragmented mRNA (green trace) fractions by CGE analytics.



Initial sample is presented with red trace.

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The CGE results demonstrate that the collected fronting fraction of the initial sample contains mainly fragments (green trace), and fraction of the main peak is predominantly intact mRNA (blue trace).

mRNA LONG-TERM STABILITY STUDY

The aim of the study is to assess the stability of the mRNA analog standard (500 ± 10 µg/mL in 1 mM sodium citrate pH 6.4) at four different temperatures and determine the storage and shipping conditions for this product. Aliquots of mRNA analog standard were stored for 6 months at four different temperatures: -80 °C, -20 °C, 4–8 °C and 20–25 °C. Stability after 15 freeze-thaw cycles was also tested.

The freeze-thaw sample has gone through 15 cycles of the freeze-thaw sample handling process. The sample was stored at -80 °C between cycles.

Chromatograms in Figures 11 & 12 present the results of the stability study of mRNA analog standard (mFix4) at the initial point and after 6 months, stored at different conditions, analyzed by SDVB analytics. In Figures 13 & 14 electropherograms of the same samples analysed by CGE analytics are presented.

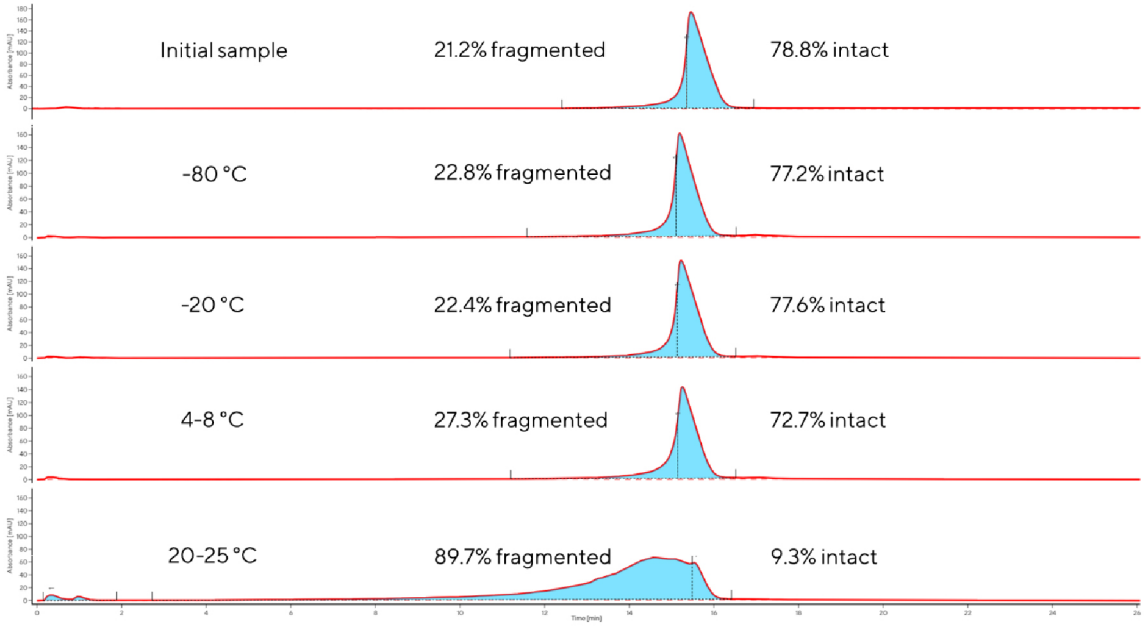
After 6 months of mRNA storage, the peak profile does not significantly change (less than 1 percentage point) from the initial (21.2% fragmentation) when mRNA is stored at -80 °C, -20 °C, and after 15 freeze-thaw cycles. After 6 months at -80 °C, SDVB analytics estimates the fragmentation at 23%, while the orthogonal CGE method estimates the fragmentation at 27%, resulting in a 4 percentage point difference in degradation estimation. The initial sample was not measured on CGE as the method was not fully implemented at the start of the study.

Comparable results were observed after 6 months at 20 °C, where SDVB analytics estimates 23% of fragmentation and orthogonal CGE analytics evaluate fragmentation at 28%. A difference of 5 percentage points comparing both analytics is observed.

With storage at a higher temperature of 4–8 °C slight degradation was observed. Here the results of the two analytics differ noticeably, as the CGE estimates mRNA

FIGURE 11

Stability results of mRNA analog standard at time zero and after 6 months, stored at four different temperatures using SDVB analytics at UV 260 nm.



fragmentation at a higher 40% compared to the 28% estimated by the SDVB analytics. This is yet to be investigated but is, in any case, a surprising result due to the demonstrated strong agreement of both analytics for other samples.

Higher degradation is observed with the sample stored at room temperature for 6 months.

FIGURE 12

Extent of fragmentation of the mRNA analog standard after 15 freeze-thaw cycles (15 x F/T), analyzed by SDVB analytics at UV 260 nm.

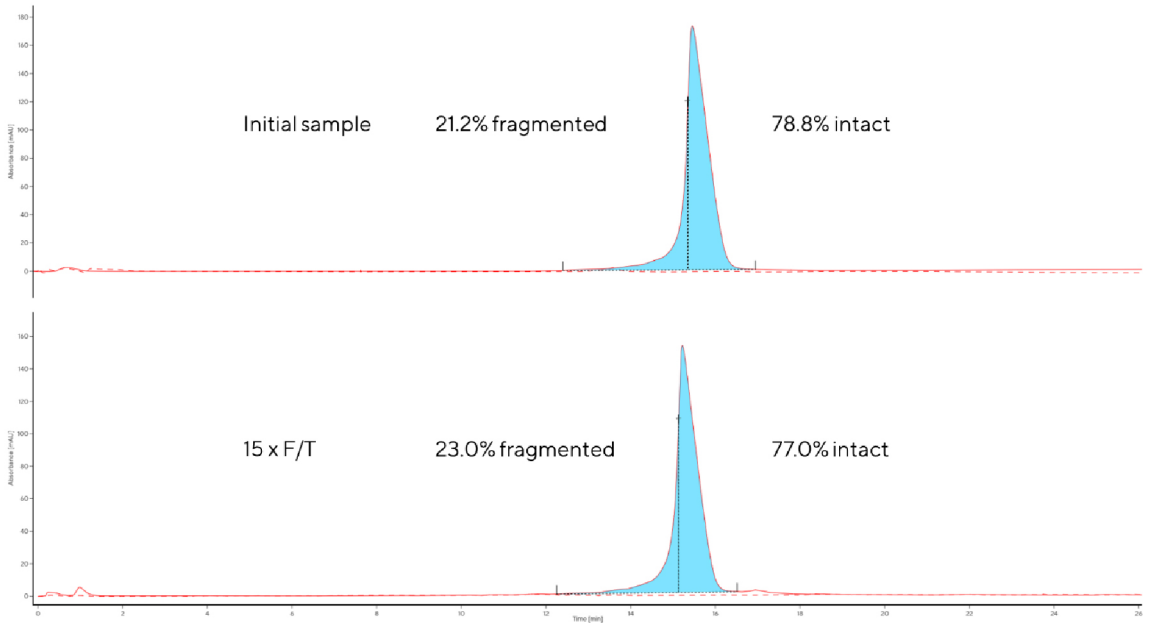
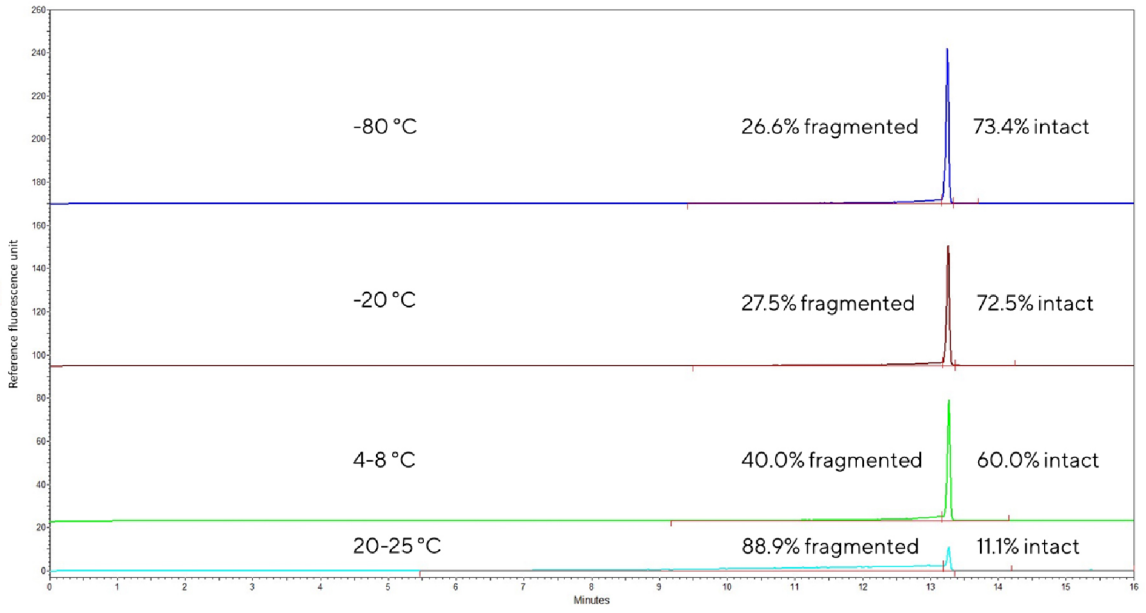


FIGURE 13

Extent of fragmentation of the mRNA analog standard after 6 months, stored at four different temperatures using CGE analytics.



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In this case, most of the sample is degraded, and the result is confirmed by the CGE.

mFix4 standard after 15 cycles of freeze-thaw was tested on SDVB analytics, where 23% of fragmentation was observed. Orthogonal CGE analytics estimated fragmentation at 31.2%, resulting in an 8 percentage points difference between the two analytics.

Orthogonal CGE results support SDVB analytics results with a difference of less than 8 percentage points at all except one temperature checkpoint. The observed increase in fragmentation by CGE analytics can be explained by the fact that these analytics were conducted subsequently to the SDVB analytics and therefore this slight delay could have an impact on the extent of fragmentation. All the data available confirm that SDVB analytics can be employed as an effective analytical approach for sample characterization (Table 3).

HIGH TEMPERATURE STABILITY OF mRNA SAMPLE

IP-RP HPLC chromatography is an established method for RNA stability assessment [8] and is often performed at elevated temperatures [8,9]. The elevated temperature enhances the resolution of oligonucleotides and RNA molecules [10] and therefore the SDVB analytical method was set to an elevated temperature of 60 °C.

The production batch of the mRNA analog standard used in this study differed from the one used in the long-term stability study, resulting in different percentages of fragmentation of the initial sample.

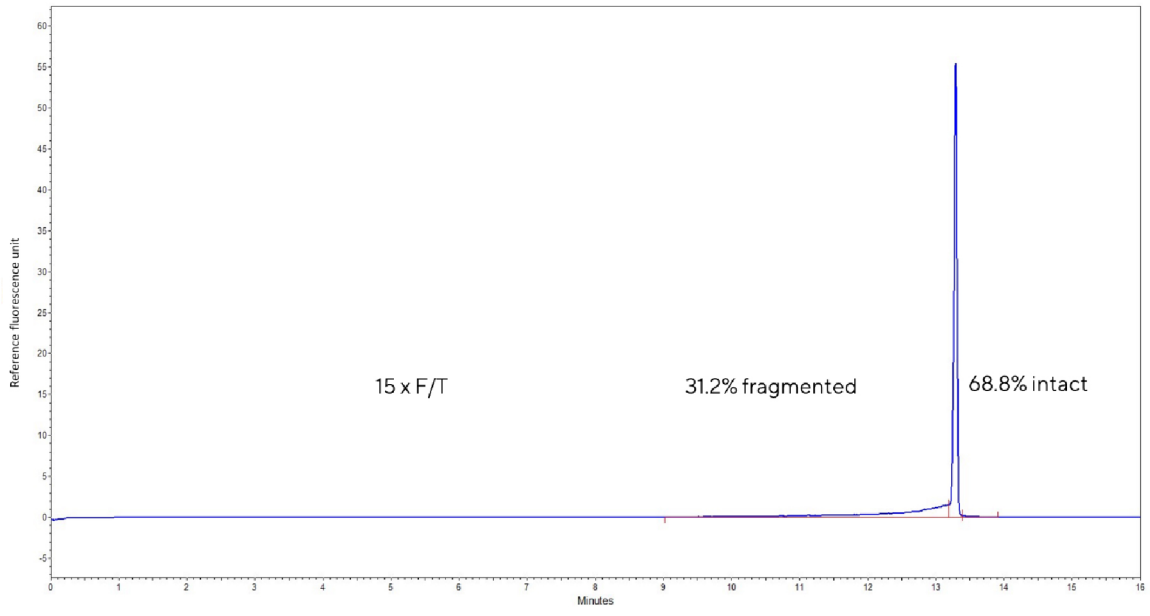
To assess the stability of the mRNA sample during the SDVB analytical run, an incubation at 60 °C was carried out. Temperature study was performed in triplicates using a Thermo-Shaker, with the sample prepared in RNase-free ultra-pure water. To confirm the SDVB results, an orthogonal CGE analysis was performed.

RNA fragmentation of the samples treated for 5, 15, 30, 45, and 60 min at 60 °C was determined by SDVB and CGE analytics (Figure 15).

The primary objective of the study was to determine whether the mRNA sample could maintain its structural integrity for a minimum duration of 30 min (retention time of

FIGURE 14

Extent of fragmentation of the mRNA analog standard after 15 freeze-thaw cycles (15 x F/T), analyzed by CGE analytics.



mRNAs on the SDVB column is 15 min) at the elevated temperature of 60 °C.

Fragmentation of the initial sample determined by SDVB and CGE analytics was 32±2% (Table 4). After 15 min at 60 °C the determined fragmentation by SDVB and CGE analytics increases by 3 and 2 percentage points, respectively. The rate of fragmentation, as assessed by either analytical technique, demonstrates a close match.

As can be seen in Figure 15, after 30 min only slightly increased fragmentation (3 percentage points) was observed with either analytical technique, indicating that during the

TABLE 3

Comparison of mRNA degradation after 6 months at different temperatures using SDVB and CGE analytics.

Temperature/°C	% fragmentation SDVB	% fragmentation CGE
Initial sample	21.2	/*
−80	22.8	26.6
−20	22.4	27.5
4–8	27.3	40.0
20–25	89.7	88.9
15 F/T	23.0	31.2

*Not measured. CGE: Capillary gel electrophoresis; SDVB: Styrene-divinylbenzene.

FIGURE 15

Percentage of mRNA fragmentation at 60 °C monitored by SDVB and CGE analytics.

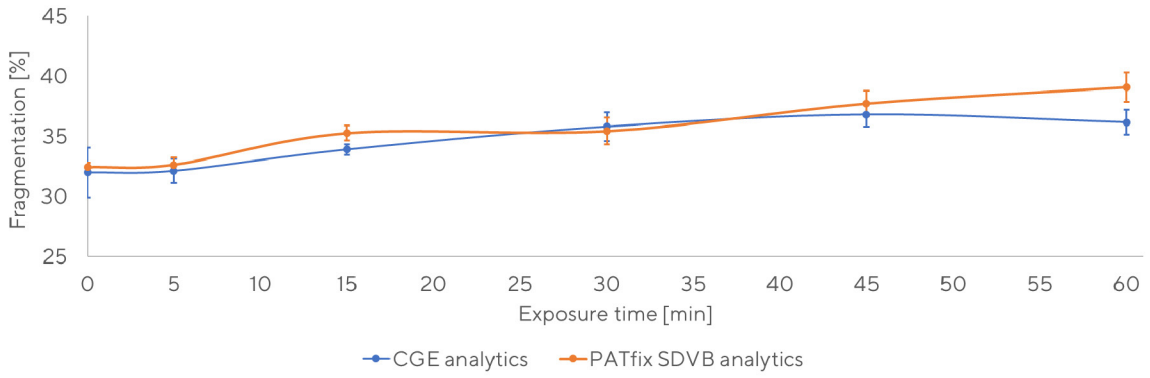


TABLE 4

Comparison of average mRNA fragmentation at 60 °C for 60 min monitored by SDVB and CGE analytics.

Time/min	SDVB analytics		CGE analytics	
	Average/%	RSD	Average/%	RSD
0	32	0.4	32	2.1
5	33	0.6	32	1.0
15	35	0.6	34	0.4
30	35	1.1	36	1.2
45	38	1.1	37	1.0
60	39	1.2	36	1.0

CGE: Capillary gel electrophoresis; RSD: Relative standard deviation; SDVB: Styrene-divinylbenzene.

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SDVB analytical run the mRNA fragmentation degree is very low.

After 60 min mRNA fragmentation exhibited 7 percentage points increase as assessed by SDVB analytics, and CGE analytics calculated 4 percentage points increase, demonstrating a minor variance between the two analytical methods.

ELEVATED TEMPERATURE mRNA STABILITY

In this experiment, the mRNA sample was incubated at 60 °C, and aliquots were sampled after 1, 2, 6, 9, 12, 18 and 24 h. Sample degradation, observed as peak fronting, was estimated at every time point with SDVB (Figure 16) and CGE (Figure 17) analytics.

The initial sample analyzed by SDVB analytics was found to be 28.5% fragmented, while CGE analytics estimated starting fragmentation at 28.7%, resulting in a 0.2 percentage point difference.

After one hour, at 60 °C the fragmentation determined by SDVB is 35.5%, showing a 7 percentage points increase in fragmentation, presenting a slight increase in sample degradation. Findings are consistent with the results from the 60 min degradation study.

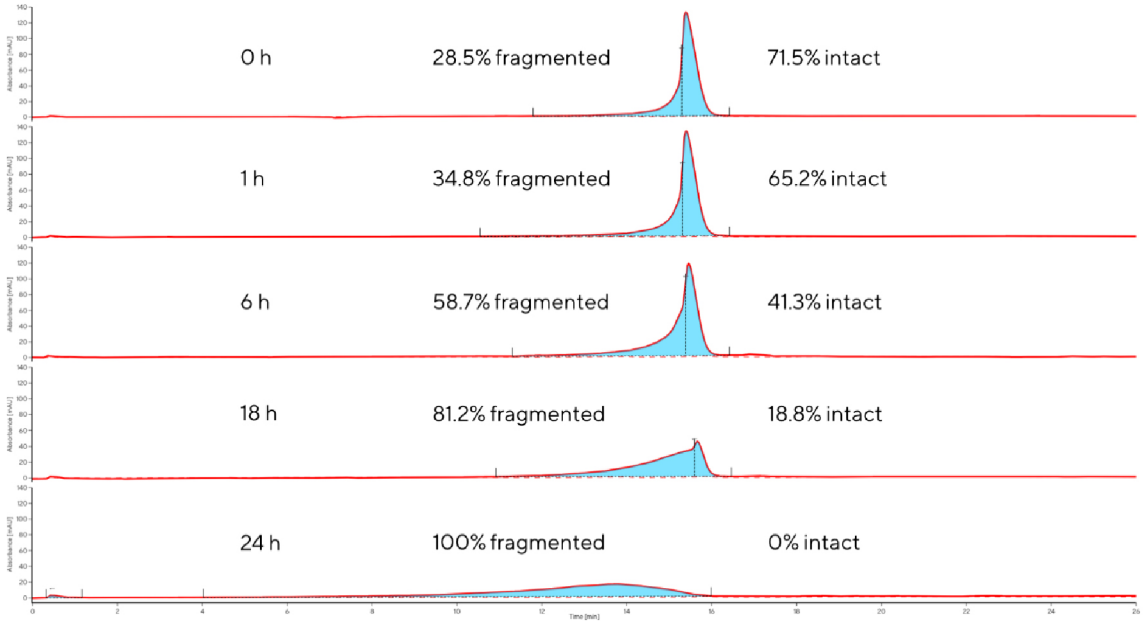
Sample fragmentation further increases at longer contact times, resulting in increased peak fronting. After 24 h, the sample is almost completely degraded. The results from the two analytical methods are comparable (Figure 18).

SDVB ANALYTICAL METHOD OFFERS A SOLUTION FOR mRNA FRAGMENTATION ASSESSMENT

This SDVB analytical method is a comprehensive characterization technique within a single chromatographic run. This approach not only facilitates the effective separation of IVT-based impurities from mRNA but also enables the identification of RNA-based impurities, such as dsRNA and RNA fragments. Given that dsRNA is a prominent impurity with the potential to induce immunogenic responses in patients, its control is crucial [2]. This dual capability of the chromatographic method not only accelerates QC

FIGURE 16

Percentage of fragmentation of mRNA at different times at 60 °C monitored by SDVB analytics.



testing but also offers a comprehensive approach, adding significant value compared to CGE analytics.

The SDVB method selectivity was demonstrated by collecting fronting and main peak of the mRNA sample. Both analytics confirm that fronting of the mRNA, observed on SDVB analytics, contains predominantly fragmented mRNA, and main peak consists of mainly intact mRNA. The SDVB analytical method can be used for estimation of percentage of fragmentation in an unknown sample.

To showcase the applicability of the SDVB method for mRNA fragmentation assessment, a 6-month stability study was conducted. mRNA analogue standard was stored at four temperatures: -80 °C, -20 °C, 4–8 °C, and 20–25 °C. The study also investigated the impact of 15 freeze-thaw cycles.

FIGURE 17

Percentage of fragmentation of mRNA at various times at 60 °C monitored by CGE.

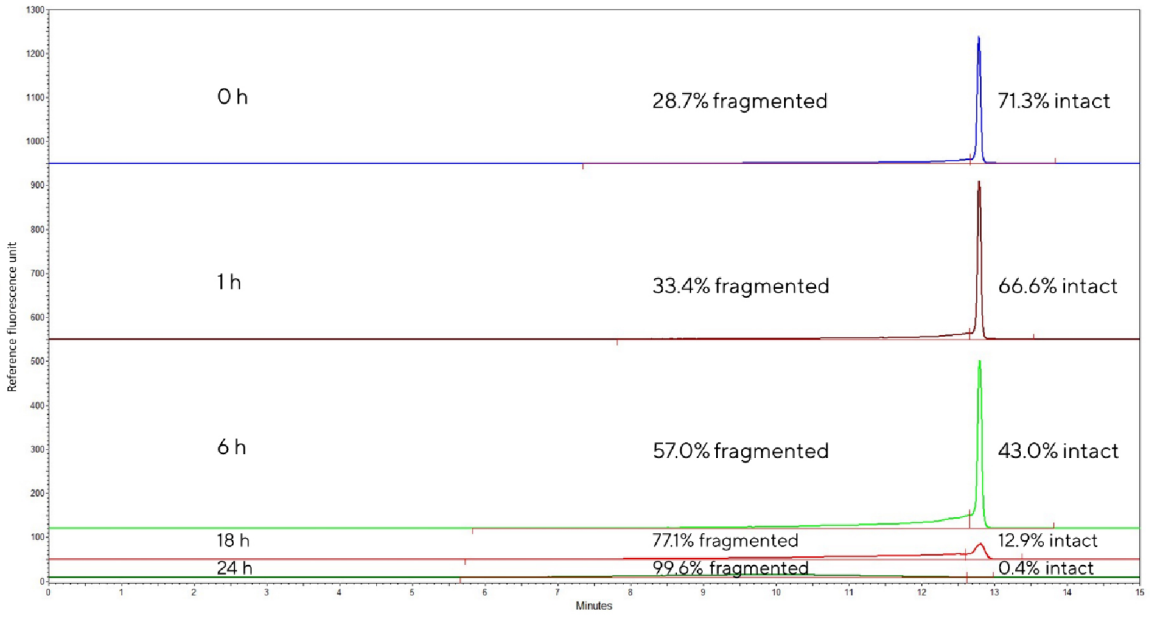
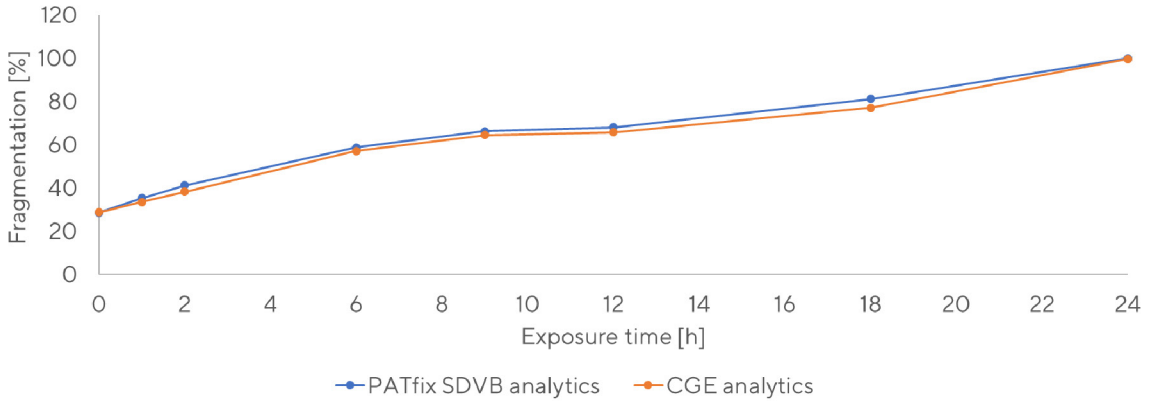


FIGURE 18

Percentage of fragmentation of mRNA at different exposure times at 60 °C monitored by PATfix SDVB and CGE analytics.



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Results obtained by the SDVB analytics show no significant degradation in the samples stored in the freezer at both -80 °C and -20 °C after the 6-month period. Orthogonal CGE analytics supports the findings as a maximum difference between analytics of 5 percentage points is observed. With this experiment, long-term stability of the mFix4 sample at -80 °C and 20 °C for at least 6 months was proved.

After 6 months at a higher temperature of 4–8 °C, both analytics confirmed a rather unexpected result that only a slight fragmentation of the mRNA mFix4 occurred. Although CGE analytics estimate fragmentation at a higher percentage (40%) compared to SDVB analytics (27.3%), the sample presented unusual stability. Furthermore, the mRNA standard stability after 15 cycles of freeze-thaw was evaluated using both analytics, where an 8 percentage point difference between the two methods is observed. These results confirm and extend previous findings, showcasing increased sample stability during the chromatographic purification step in comparison to precipitation, as previously demonstrated [11].

To confirm the SDVB analytical method does not overestimate the RNA fragmentation due to possible degradation during the analytical run, the 60 min degradation study at 60 °C was performed. After 30 min the percentage of fragmentation increased by 3 and 4 percentage points as determined by SDVB and CGE analytics, respectively. Low level of sample fragmentation and the good agreement between the data from both analytics suggests that the data obtained by the SDVB method does not over- or under-estimate the percentage of fragmentation.

Comparison of the high-temperature stability for 24 h study data shows complete agreement between CGE and SDVB analytics. mFix4 sample is unexpectedly resistant to high-temperature incubation after one hour. As expected, at longer contact times and elevated temperature, the peak fronting increased, leading to higher sample fragmentation levels in both SDVB and CGE analytics. After 24 hours of incubation, the sample showed almost complete degradation, as demonstrated by an almost complete absence of the main mRNA peak.

CONCLUSION

The presented method for determining the extent of mRNA fragmentation, using the CIMac SDVB monolithic column in a PATfix chromatographic analytical platform, offers an easy-to-use tool delivering results that are robust, reliable, and in close agreement with those obtained by the CGE.

The SDVB analytical method enables characterization of complex samples independent of the sample matrix in addition to its ability to detect various contaminants such as dsRNA, DNA template, capping reagents, and nucleotides, which is not provided by the CGE analytics. This study demonstrates the importance of robust analytical methods for mRNA product development and quality control, bolstering a safe and effective advancement of mRNA-based therapies and vaccines.

REFERENCES

1. Wei HH, Zheng L, Wang Z. RNA therapeutics: new vaccination and beyond. *Fundamental Research* 2023; Epub ahead of print.
2. USP. *Analytical Procedures for mRNA Vaccine Quality 2nd Edition.* 2022; BioProcess Int.
3. Budihna AF, Pavlin N, Celjar AM, Livk AG, Strancar A. *High-Selectivity Biochromatography mRNA Analytics.* 2022; BioProcess Int.
4. Yang TH, Chang PO. Determination of RNA degradation by capillary electrophoresis with cyan light-emitted diode-induced fluorescence. *J. Chromatogr. A* 2012; 1239, 78–84.
5. Raffaele J, Loughney JW, Rustandi RR. Development of a microchip capillary electrophoresis method for determination of the purity and integrity of mRNA in lipid nanoparticle vaccines. *Electrophoresis* 2022; 43, 9–10.

6. Bidlingmeyer BA, Warren FV. Column efficiency measurement. *Anal. Chem.* 1984; 56(14), 1583A–1596A.

7. Wahab MF, Patel DC, Wimalasinghe RM, Armstrong DW. Peak shapes and their measurements: the need and the concept behind total peak shape analysis. *J. Chromatogr. A* 2017; 1509, 163–170.

8. Azarani A, Hecker KH. RNA analysis by ion-pair reversed-phase high performance liquid chromatography. *Nucleic Acids Res.* 2001; 29(2), E7.

9. Packer M, Gyawali D, Yerabolu R, *et al.* A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. *Nat. Commun.* 2021; 12(1), 6777.

10. Huber CG, Oefner PJ, Bonn GK. High-resolution liquid chromatography of oligonucleotides on non-porous alkylated styrene-divinylbenzene copolymers. *Anal. Biochem.* 1993; 212(2), 351–358.

11. Korenč M, Mencin N, Puc J, *et al.* Chromatographic purification with CIMmultus™ Oligo dT increases mRNA stability. *Cell Gene Ther. Insights* 2021; 7(9), 1207–1216.

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