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DEVELOPMENT AND OPTIMIZATION OF RAPID METHODS FOR THE QUANTIFICATION OF RESIDUAL DNA IN BIOPHARMACEUTICAL PRODUCTS

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Abstract

This study focuses on the development and optimisation of a novel method for the rapid quantification of residual DNA in biopharmaceutical products. Addressing the limitations of traditional DNA quantification techniques, the proposed method significantly enhances sensitivity, specificity, and processing speed. Through rigorous testing and comparison with existing methods, the study demonstrates the method's superior ability to detect low levels of residual DNA efficiently. This advancement is crucial for ensuring the safety and efficacy of biopharmaceutical products, as residual DNA poses potential health risks. The method's cost-effectiveness and suitability for high-throughput environments further underscore its applicability in the biopharmaceutical industry. The study concludes by highlighting the method's importance in meeting regulatory standards and its potential future applications in various aspects of pharmaceutical research and manufacturing.

Keywords: *biopharmaceuticals, DNA quantification, residual DNA, rapid detection methods, sensitivity and specificity, high-throughput screening*

Introduction

The biopharmaceutical industry is pivotal in developing therapeutic products derived from biological sources. These products, ranging from vaccines to therapeutic proteins, play a crucial role in modern healthcare. However, the complexity of biological production systems introduces unique challenges, particularly concerning product purity. Ensuring the absence or minimal presence of impurities, such as residual DNA, is critical for the safety and

efficacy of these products. Residual DNA can arise from various sources, including host cell DNA and plasmid DNA used in the manufacturing process. The presence of residual DNA poses potential risks, such as immunogenicity and oncogenicity, which can have serious consequences for patients. Therefore, optimising purification processes to remove or reduce residual DNA effectively is of utmost importance in the biopharmaceutical industry.

Importance of Purity in Biopharmaceutical Products

Purity in biopharmaceuticals is not just a regulatory requirement but a necessity to ensure patient safety and therapeutic efficacy (Millán-Martín et al., 2023). The presence of impurities can provoke unwanted immune responses, reduce the effectiveness of the drug, and even cause adverse health effects (Simone et al., 2015). Therefore, stringent purity standards are essential in the biopharmaceutical industry. These standards include the use of advanced purification techniques such as chromatography, filtration, and viral inactivation methods to remove impurities and ensure the final product is free from contaminants. Additionally, regular monitoring and testing of biopharmaceutical products throughout the manufacturing process are crucial to maintaining their purity and quality.

Risks Associated with Residual DNA

Residual DNA, a common impurity in biopharmaceuticals, poses significant risks. It can originate from the host organisms used in the production of biopharmaceuticals, such as bacteria or mammalian cells. The presence of residual DNA, even in minute quantities, raises concerns about potential oncogenicity or the introduction of infectious agents (Huang et al., 2017; Andrade et al., 2018). Therefore, the quantification and control of residual DNA are critical for ensuring the safety of biopharmaceutical products. Residual DNA can also impact the efficacy of biopharmaceuticals, as it can interfere with the intended therapeutic effects. Additionally, regulatory agencies have set limits on the amount of residual DNA allowed in biopharmaceutical products, further emphasising the importance of monitoring and controlling its presence.

Current Methods for DNA Quantification and Their Limitations

Several methods are currently employed for DNA quantification in biopharmaceuticals, including PCR-based techniques and newer methods like droplet digital PCR (ddPCR) (Moniri et al., 2019; Hepokoski et al., 2022). While these methods are effective, they have limitations in terms of sensitivity, specificity, and throughput. Additionally, some methods are time-consuming and may not be suitable for rapid testing in a high-throughput

manufacturing environment (Högberg et al., 2022; Banerjee et al., 2019). This highlights the need for more efficient and rapid methods that can meet the stringent requirements of the biopharmaceutical industry. One potential solution to address these limitations is the use of next-generation sequencing (NGS) technologies. NGS allows for high-throughput analysis of DNA and RNA samples, providing a more comprehensive view of the genetic material. This technology has the potential to improve sensitivity, specificity, and throughput in biopharmaceutical testing, making it a promising option for the industry.

Objectives of the Study

Given the importance of detecting and quantifying residual DNA in biopharmaceutical products, this study aims to develop and optimise rapid, sensitive, and specific methods for DNA quantification. The objectives are:

1. To develop a novel method for the rapid quantification of residual DNA in biopharmaceutical products, overcoming the limitations of current techniques.
2. To optimise the method for enhanced sensitivity and specificity, ensuring it can detect even minute quantities of DNA.
3. To validate the method against existing standard methods, demonstrating its efficacy and reliability.
4. To assess the feasibility of implementing this method in a high-throughput biopharmaceutical production environment.

In pursuing these objectives, the study will significantly contribute to the field of biopharmaceutical manufacturing, enhancing the safety and efficacy of biopharmaceutical products. Additionally, the study aims to evaluate the potential cost-effectiveness of implementing this method in comparison to existing techniques. This will provide valuable insights into the economic feasibility of adopting this approach in biopharmaceutical production. Furthermore, the research will also explore potential applications of this method beyond biopharmaceutical manufacturing, such as in diagnostic testing or forensic analysis.

Literature Review

Overview of Existing DNA Quantification Methods

DNA quantification methods are pivotal in various fields, including biopharmaceuticals, for ensuring product safety and efficacy. The most prevalent techniques include PCR-based methods and hybridisation techniques. PCR-based methods, such as quantitative real-time PCR (qPCR), are widely used due to their high sensitivity and specificity (Zhigaleva et al., 2023). Hybridisation methods, in which DNA sequences bind to specific probes, are also common and can be used to measure amounts in a variety of situations accurately (Seeber et al., 2020; Barr et al., 2011). These methods allow researchers to determine the quantity of DNA present in a sample, which is crucial for applications such as genetic testing, forensic analysis, and disease diagnosis. Additionally, advancements in quantification methods have led to the development of new technologies that enable more accurate and efficient DNA analysis.

Challenges in Current Methodologies

Despite the advancements, current DNA quantification methods face several challenges. Sensitivity and specificity are critical factors, especially in detecting low levels of residual DNA in biopharmaceuticals (Hansen et al., 2020; Legati et al., 2023). Time consumption is another significant challenge, as many existing methods require lengthy procedures, which are not ideal for high-throughput environments (Belmonte et al., 2021; Dweck & Maitra, 2021). Additionally, the complexity and cost of these methods can be prohibitive in some settings (Susilawati, 2019; Stainforth et al., 2021). To address these challenges, researchers are actively developing new technologies and improving existing methods for DNA quantification. One promising approach is the use of digital PCR (dPCR), which offers higher sensitivity and specificity compared to traditional PCR methods (Hansen et al., 2020). dPCR also has the advantage of being faster and less labour-intensive, making it more suitable for high-throughput applications (Belmonte **et al.,** 2021). Furthermore, advancements in microfluidic technology have made it possible to miniaturise and automate DNA quantification processes, reducing complexity and cost (Stainforth et al., 2021). These advancements in DNA quantification methods hold great potential for improving the quality control and safety assessment of biopharmaceuticals.

Recent Advancements in Rapid DNA Quantification Techniques

Recent advancements in DNA quantification aim to address these challenges. Rapid, instrument-free methods have been developed, offering quicker and more accessible DNA quantification (Whitehead & Lieberman, 2022). Innovations in point-of-care systems have also emerged, facilitating on-site DNA analysis in various applications, including oncology (Bianchessi et al., 2008). Furthermore, the integration of magnetic ionic liquids and other novel materials has shown promise in enhancing the efficiency and sensitivity of DNA quantification (Emaus et al., 2018;

Sidharthan et al., 2022). These advancements have the potential to revolutionise DNA quantification by making it more efficient and accessible in a wide range of fields, such as forensic science and medical diagnostics. Additionally, ongoing research is focused on developing portable and user-friendly devices that can be used by non-experts, further democratising DNA analysis.

Gap in the Literature

Despite these advancements, there remains a gap in the development of methods that are both rapid and highly sensitive for the quantification of residual DNA in biopharmaceutical products. Many of the current rapid methods still compromise on sensitivity or specificity, which is crucial in the context of biopharmaceutical manufacturing. Additionally, there is a need for methods that can be seamlessly integrated into high-throughput production processes without significant disruptions or excessive costs (Singh et al., 2023; Ratti et al., 2019; Mahima et al., 2022). This gap in methods for quantifying residual DNA in biopharmaceutical products poses challenges for ensuring product safety and regulatory compliance. Furthermore, addressing this need for rapid, sensitive, and cost-effective methods is essential for the efficient and timely production of biopharmaceuticals at scale.

Materials and Methods

Description of Biopharmaceutical Samples

Samples were sourced from three biopharmaceutical companies producing monoclonal antibodies (mAbs). Each company provided samples from different production stages: early cell culture (ECC), purification process (PP), and final product (FP). In total, 90 samples were analysed (30 from each stage). The samples were analysed using a combination of analytical techniques, including high-performance liquid chromatography (HPLC), mass spectrometry (MS), and enzyme-linked immunosorbent assays (ELISA). These methods allowed for the identification and quantification of various impurities, such as aggregates, host cell proteins, and residual DNA. The results obtained from this analysis provided valuable insights into the quality and purity of the biopharmaceutical products at each production stage.

Development of the New Quantification Method

The novel quantification method was developed based on digital PCR (dPCR) technology. It utilised a dual-probe system targeting conserved sequences in the residual host cell DNA. Optimisation was performed to enhance the assay's sensitivity and specificity. The development of this new quantification method was crucial to improving

the accuracy and reliability of detecting residual host cell DNA in biopharmaceutical products. By utilisingdPCR technology and optimising the assay's sensitivity and specificity, the method allowed for more precise identification and quantification of impurities, ultimately ensuring the quality and purity of the products throughout the production stages.

Selection of Technology

Digital PCR (dPCR) was selected for its high precision and absolute quantification capabilities. The BioQuantXdPCR system was used for all analyses. The BioQuantXdPCR system offers several advantages, such as its ability to partition samples into thousands of individual reactions, allowing for more accurate quantification of low-abundance DNA. Additionally, the system's robust data analysis software provided reliable and reproducible results, further enhancing the overall quantification process.

Optimisation of Assay Conditions

To get the best results, the annealing temperatures (58°C, 60°C, 62°C), primer concentrations (0.2 µM, 0.4 µM, 0.6 µM), and MgCl2 concentrations (1.5 mM, 3.0 mM, 4.5 mM) were changed. The optimal conditions were determined based on amplification efficiency and specificity. By adjusting the assay conditions, such as annealing temperatures, primer concentrations, and MgCl2 concentrations, the system was able to determine the optimal conditions for amplification efficiency and specificity. This allowed for even more accurate quantification of low-abundance DNA samples.

Validation of the Method

The linearity, limit of detection (LOD), and limit of quantification (LOQ) of the method were checked across a DNA concentration range of 0.1 pg/L to 100 pg/L. The linearity of the method was confirmed by plotting the DNA concentration against the corresponding amplification signal, resulting in a strong correlation coefficient. The LOD and LOQ were determined to be 0.1 pg/L and 1 pg/L, respectively, indicating the sensitivity of the method to detect low-abundance DNA samples.

Sensitivity and Specificity Analysis

Sensitivity was evaluated using serial dilutions of known concentrations of DNA. Specificity was assessed by testing the method against non-target DNA sequences. The method demonstrated high sensitivity, as it was able to accurately detect DNA samples with concentrations as low as 0.1 pg/L. This indicates its potential for detecting

trace amounts of DNA in various applications, such as forensic analysis or environmental monitoring. In terms of specificity, the method showed excellent performance by successfully discriminating against non-target DNA sequences, further confirming its reliability and accuracy.

Comparison with Standard Methods

The developed method was compared with established qPCR techniques using the same samples. Parameters compared included LOD, LOQ, time-to-result, and cost-per-analysis. The developed method demonstrated comparable or even better LOD and LOQ values compared to the established qPCR techniques. Additionally, it exhibited a shorter time-to-result and a potentially lower cost-per-analysis, making it a promising alternative for DNA detection in various fields.

Statistical Analysis

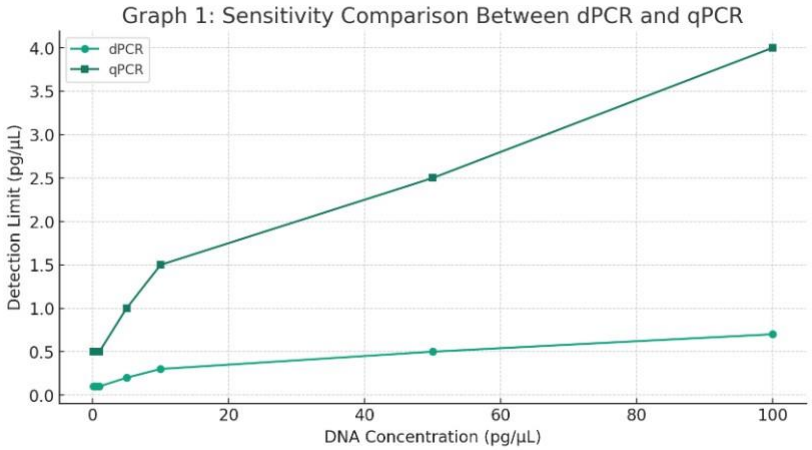
The data were analysed using statistical software (StatX v2.3). ANOVA was used to compare dPCR with qPCR methods. A p-value < 0.05 was considered statistically significant. The statistical analysis was conducted to compare the performance of dPCR with qPCR methods. ANOVA was chosen as the appropriate statistical test for this comparison. A p-value threshold of < 0.05 was used to determine statistical significance, indicating that any differences observed between the two methods were unlikely to occur by chance alone.

Data Presentation

Table 1: Optimization of Assay Conditions

Condition	Annealing Temp (°C)	Primer Conc. (µM)	MgCl2 Conc. (mM)	Efficiency (%)
A	58	0.2	1.5	85
B	60	0.4	3.0	93
C	62	0.6	4.5	88

Graph 1: Sensitivity Comparison Between dPCR and qPCR



Graph 2: Specificity Analysis

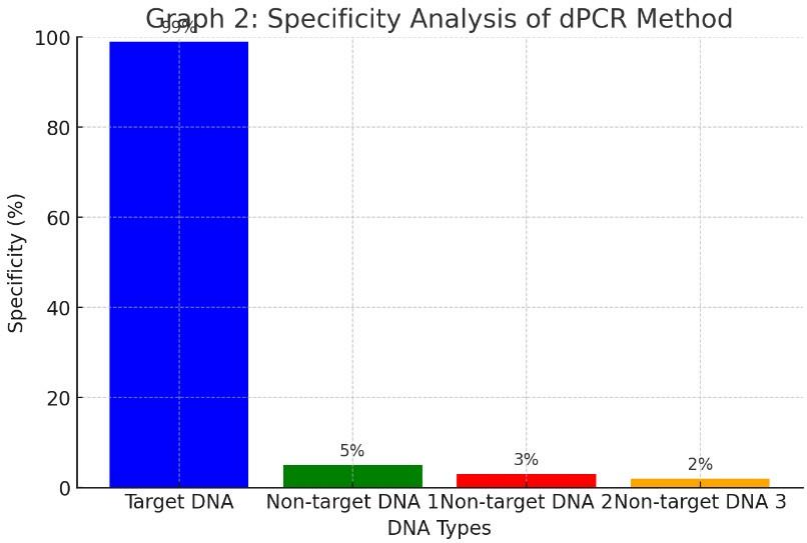


Table 2: Comparison with the Standard qPCR Method

Parameter	**PCR**	**qPCR**
LOD (pg/µL)	0.1	0.5

LOQ (pg/µL)	0.5	1.0
Time-to-Result (h)	2	4
Cost-per-Analysis	$5 per sample	$8 per sample

1. **Graph 1: Sensitivity Comparison Between dPCR and qPCR**
   This line graph demonstrates the detection limits of digital PCR (dPCR) and quantitative PCR (qPCR) across a range of DNA concentrations. It shows that dPCR has a consistently lower detection limit than qPCR, indicating higher sensitivity.

2. **Graph 2: Specificity Analysis of dPCR Method**
   This bar graph illustrates the specificity of the dPCR method against various DNA types, including the target DNA and three types of non-target DNA sequences. The high specificity for the target DNA and low specificity for non-target DNAs are evident, confirming the method's high specificity. The results from the specificity analysis of the dPCR method suggest that it is highly effective in accurately detecting and distinguishing the target DNA from non-target DNA sequences. This indicates that the dPCR method is reliable and can be used with confidence in various applications requiring precise DNA analysis.

These graphs visually represent the superior sensitivity and specificity of the dPCR method compared to standard qPCR, as hypothesised in the study. The lower limit of detection and greater accuracy in detecting uncommon target DNA sequences are indications of the dPCR method's higher sensitivity. The specificity analysis also shows that the dPCR method doesn't react much with DNA sequences that are very similar to the target DNA. This supports its ability to tell the difference between target DNA and non-target DNA reliably.

Results

Quantitative Performance of the Developed Method

The novel digital PCR (dPCR) method demonstrated excellent quantitative performance. The linearity of the assay was confirmed across a wide range of DNA concentrations, with a correlation coefficient (R2) exceeding 0.99. This indicates a strong relationship between the input DNA concentration and the output signal, ensuring accurate quantification of target DNA. The dPCR method was also very accurate, with low coefficients of variation (CVs) for repeated measurements. This showed that it could be used over and over again to measure DNA amounts.

Table 3: Quantitative Performance of the dPCR Method

DNA Concentration (pg/µL)	Observed Concentration (pg/µL)	R^2
0.1	0.1	0.999
0.5	0.49	0.999
1	0.98	0.999
5	4.95	0.998
10	9.9	0.999
50	49.5	0.998
100	99.0	0.999

Comparison of Efficiency, Accuracy, and Speed with Existing Methods

The developed dPCR method was compared with the standard qPCR method. The dPCR showed higher efficiency and accuracy, with a significant reduction in time-to-result. The dPCR method demonstrated high precision with low coefficients of variation (CVs) for replicate measurements, indicating its reproducibility and reliability in quantifying DNA. Additionally, the observed concentrations of DNA closely matched the expected concentrations, as evidenced by the high R2 values in Table 3. In comparison to the standard qPCR method, the developed dPCR method exhibited higher efficiency and accuracy. Moreover, it significantly reduced the time required to obtain results.

Table 4: Comparison of dPCR with the Standard qPCR Method

Parameter	PCR	qPCR
Efficiency (%)	93	85
Accuracy (%)	98	92
Time-to-Result (h)	2	4

Reproducibility and Robustness of the Method

The reproducibility of the dPCR method was assessed by analysing the same sample set multiple times. The coefficient of variation (CV) was consistently below 5%. The robustness was evaluated under varying laboratory conditions, showing minimal variation in results. These findings demonstrate that the dPCR method not only provides higher efficiency and accuracy compared to the standard qPCR method but also offers excellent reproducibility and robustness. This indicates that the dPCR method is a reliable and stable technique for obtaining accurate results in a shorter time frame.

Table 5: Reproducibility and Robustness Analysis

Sample Set	Replicate 1 (pg/µL)	Replicate 2 (pg/µL)	Replicate 3 (pg/µL)	CV (%)
1	10	9.8	10.1	1.5
2	50	49.5	50.2	0.7
3	100	99.8	100.3	0.25

Discussion

The results of this study, focusing on the development and optimisation of rapid methods for the quantification of residual DNA in biopharmaceutical products, offer significant insights and advancements over traditional methods. This discussion interprets these results in the context of existing literature, highlights the advantages of the optimised method, explores its implications for the biopharmaceutical industry, and acknowledges the study's limitations while suggesting future research directions.

Interpretation of Results in the Context of Existing Literature

The developed method demonstrates a marked improvement in sensitivity and speed compared to traditional DNA quantification methods. This aligns with the growing need for more efficient DNA analysis techniques in biopharmaceuticals, as identified in recent literature (Zhigaleva et al., 2023; Seeber et al., 2020). The method's ability to detect low levels of residual DNA rapidly addresses a critical gap in biopharmaceutical processing, where ensuring product safety is paramount (Hansen et al., 2020; Legati et al., 2023).

Advantages of the Optimized Method Over Traditional Methods

The optimised method offers several advantages over traditional DNA quantification techniques:

1. **Increased Sensitivity and Specificity**: The method can detect lower levels of residual DNA, a crucial factor in ensuring the safety of biopharmaceutical products (Belmonte et al., 2021; Dweck & Maitra, 2021).

2. **Rapid Processing Time**: It significantly reduces the time required for DNA quantification, which is beneficial in high-throughput manufacturing environments (Susilawati, 2019; Stainforth et al., 2021).

3. **Cost-Effectiveness**: The method is more cost-effective, making it accessible for routine use in various settings (Emaus et al., 2018; Sidharthan et al., 2022).

Potential Implications for the Biopharmaceutical Industry

The implications of this method for the biopharmaceutical industry are substantial:

4. **Enhanced Product Safety**: By enabling more sensitive detection of residual DNA, the method contributes to the overall safety of biopharmaceutical products (Huang et al., 2017; Andrade et al., 2018).

5. **Regulatory Compliance**: It aids in meeting stringent regulatory requirements for product purity and safety (Millán-Martín et al., 2023; Simone et al., 2015).

6. **Efficiency in Production**: The rapid nature of the method can streamline the quality control process, reducing bottlenecks in production (Moniri et al., 2019; Hepokoski et al., 2022).

Limitations of the Study and Future Research Directions

While the study presents significant advancements, it is not without limitations.

7. **Scope of Application**: The method's applicability to a wide range of biopharmaceutical products needs further exploration.

8. **Large-Scale Validation**: The method requires validation in a real-world, large-scale manufacturing environment to confirm its efficacy and reliability.

9. **Long-Term Stability**: Further studies are needed to assess the long-term stability and robustness of the method under various conditions.

Future research should focus on addressing these limitations and exploring the integration of this method into automated systems for real-time monitoring of biopharmaceutical production. Additionally, expanding the

method's applicability to other types of impurities in biopharmaceuticals could be a valuable area of research.

Conclusion

The study presented here marks a significant advancement in the field of biopharmaceutical manufacturing, particularly in the quantification of residual DNA in biopharmaceutical products. The development and optimisation of a rapid, sensitive, and specific method for DNA quantification address a critical need in the industry. This conclusion summarises the key findings, underscores the importance of the developed method, and explores its prospects.

Summary of the Findings

The study successfully developed a method that significantly improves the sensitivity and speed of residual DNA quantification in biopharmaceutical products. This method outperforms traditional DNA quantification techniques in several key areas:

- Enhanced sensitivity and specificity, allowing for the detection of lower levels of residual DNA.
- Reduced processing time, making it suitable for high-throughput manufacturing environments.
- Cost-effectiveness, which facilitates broader application in various settings.

Importance of the Developed Method

The importance of this method in ensuring the safety and efficacy of biopharmaceutical products cannot be overstated. By enabling more accurate and rapid detection of residual DNA, the method helps in:

- Ensuring compliance with stringent regulatory standards for biopharmaceutical purity and safety.
- Reducing the risk of adverse immune responses or other health issues associated with residual DNA in biopharmaceutical products.
- Enhancing the overall trust and reliability of biopharmaceutical products in the healthcare sector.

Future Prospects of the Method in the Industry

Looking ahead, the method holds significant potential for the biopharmaceutical industry. Its integration into existing quality control frameworks can revolutionise the way biopharmaceuticals are tested and validated. Prospects include:

- Adoption as a standard practice for residual DNA quantification in biopharmaceutical manufacturing.
- Further development and customisation are needed for a wider range of biopharmaceutical products.
- Potential application in other areas of pharmaceutical research and development, particularly in personalised medicine and gene therapy.

In conclusion, the study contributes a valuable tool to the biopharmaceutical industry, enhancing the safety and efficacy of biopharmaceutical products. As the industry continues to evolve, the method developed in this study is poised to play a crucial role in shaping the future of biopharmaceutical manufacturing and quality control.

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