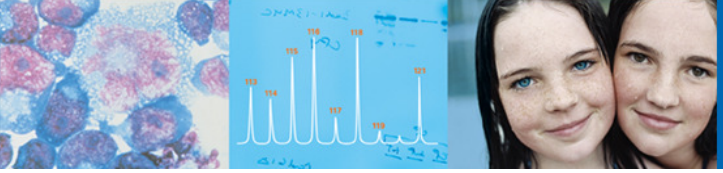

Technical Note

Analysis and Quantitation of Oligonucleotides

4800 Plus MALDI TOF/TOF™ Analyzer

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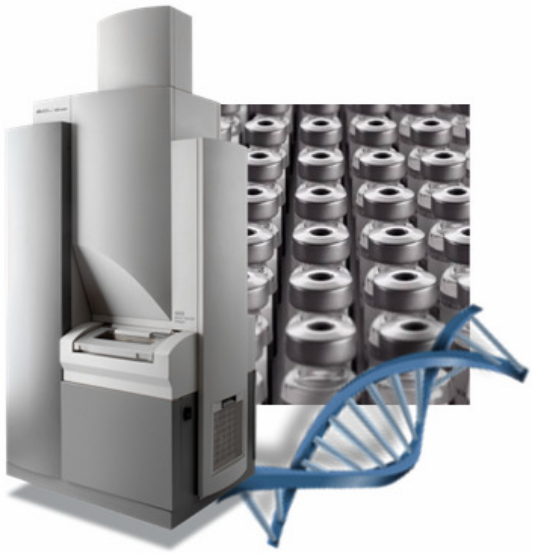

In recent years, oligonucleotides have emerged as a new class of therapeutics to treat cancer, infectious diseases, allergy and asthma. Therefore, there is an increasing need to analyze and quantitate oligonucleotides. Matrix-assisted laser desorption/ionization time of flight mass spectrometers (MALDI-TOF) has been successfully used for the analysis of oligonucleotides due to its ease of use and speed of analysis. However, quantifying oligonucleotides using MALDI-TOF proves to be more difficult due to the existence of non-homogeneity of sample distribution within a spot, or "hot spots". In this technote, we describe the use of sugar additives combined with internal standards for the quantitation of oligonucleoptides using an AB/SCIEX 4800 plus MALDI TOF/TOF™ Analyzer.

Experimental

Sample preparation: Oligonucelotide standards were purchased from Qiagen and used without any additional purification, dissolving the lyophilized oligonucleotide directly in deionized water. D-Fructose and 2',4',6'-Trihydroxyacetophenone (THAP) were purchased from Sigma-Aldrich.

THAP was dissolved in 90% ACN: 10% H_2O at a concentration of 20 mg/mL. Diammonium Hydrogen Citrate was added to the solution at a concentration of 50 mg/mL to decrease the salt adducts in the spectra. To get more uniform sample crystallization and decrease the existence of "hot spots", D-Fructose was added to the above matrix solution to a final concentration of 2 mg/mL. To get the best linearity, a 22mer oligonucleotide standard was added to the matrix solution as internal standard (132 fmol/µL).

A dilution series of the 20mer oligonucleotide standard was prepared from the stock solution. Then 2 µL of the diluted solution was mixed with 2 µL of the matrix solution and 0.5 µL was spotted onto a standard stainless steel plate.

Mass Spectrometry: The samples are analyzed in negative reflector mode using the standard acquisition methods within the mass range of 800-8000 Dalton. The laser intensity was fixed at 5600. 2500 shots were acquired at each concentration.

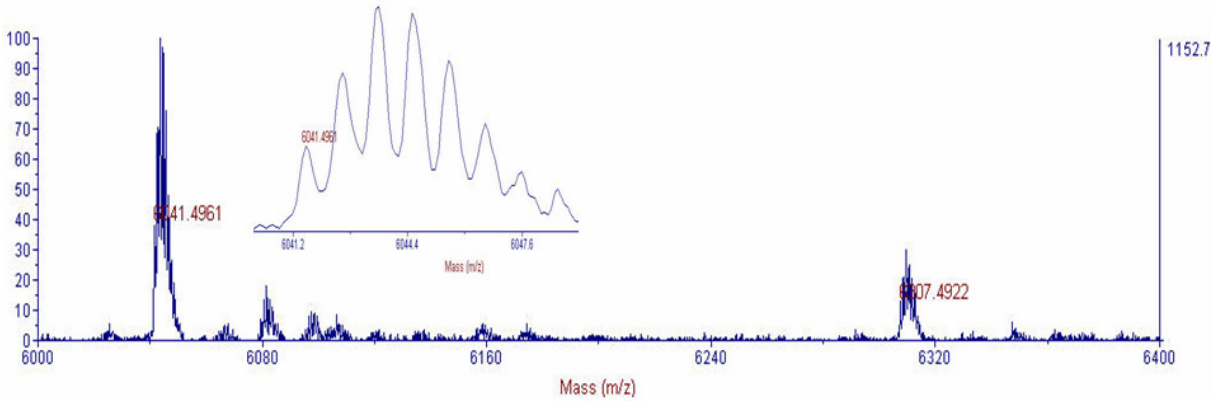

Figure 1. MS Spectra of Oligonucleotides. MS spectra was collected in reflector mode and a resolution of 19957 was obtained. Shown is the data from 70 fmol concentration, where both the analyte and internal standard are easily detected.

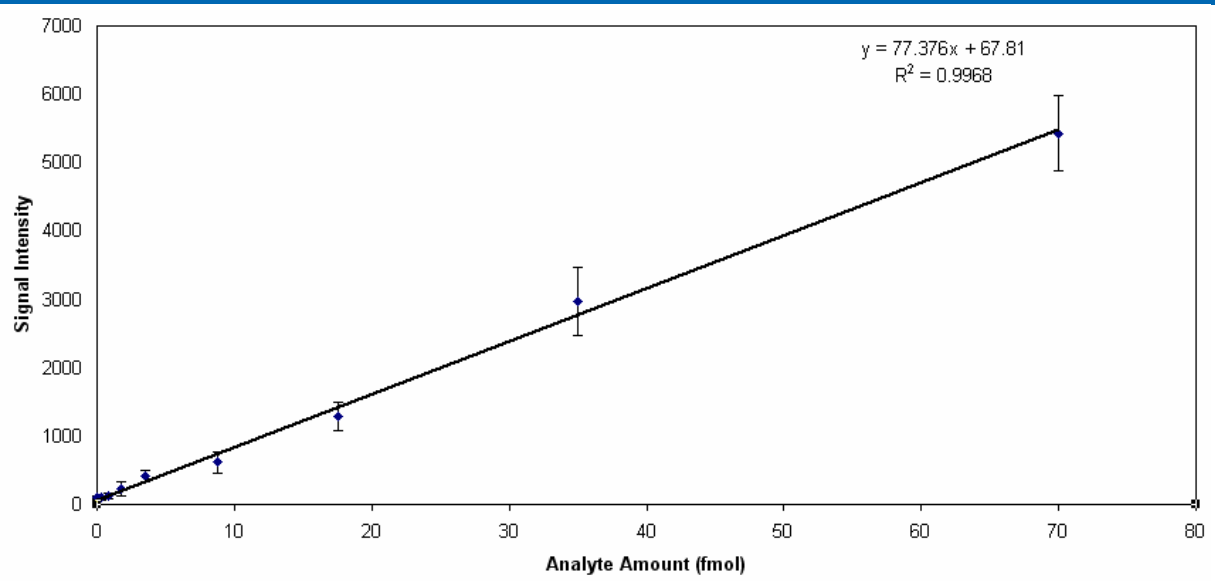

Figure 2. Standard curve for 20 mer oligonucleotide. Good linearity was obtained from 1.75 fmol to 2 pmol oligo nucleotide on spot. The lower limit of quantitation (LLOQ) obtained was 1.75 fmol showing good sensitivity for this application by MALDI TOF analysis.

Results

Figure 1 shows both the analyte (20mer) and the internal standard (22mer) can be easily detected with high resolution (R=~20000). Shahgholi *et al.*[1] reported various sugars can be used as matrix additives to enhance the sample uniformity and minimize the transfer of excess laser energy to oligonucleotides. As a result, a higher laser power can be used to ensure the generation of enough ions for quantitation without compromising the data quality.

To get the best reproducibility and minimize the effect of "hot spots", an internal standard is still necessary. In this experiment, the average peak area of the 22mer standard was used to normalize the peak area of the analytes at every concentration level. Five replicates were acquired for the analytes at each concentration level. The average normalized peak area at each concentration level was used for plotting the standard curve. The standard deviation within the same concentration is less than 18%. As shown in Figure 2, good linearity across 3 orders of dynamic range was achieved, with r value of 0.99 and the lower limit of quantitation at low fmol level.

Conclusions

The 4800 Plus MALDI TOF/TOF™ Analyzer is a robust platform for analyzing oligonucleotides. By using internal standards and matrix additives, good linearity can be achieved within 3 orders of magnitude.

References

1. Mona Shahgholi, Benjamin A. Garcia, Norman H. L. Chiu, Paul J. Heaney and Kai Tang, Nuclei Acids Research, 2001, Vol. 29, No. 19.

114TN68-01

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