

Quantification of Isomeric Oligonucleotides in Mixtures by Micro-HPLC/ESI/MS/MS and a Vector Data Processing Method

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The structure of DNA in living cells is constantly altered by endogenous and exogenous chemical, physical, and biological agents. Most enzymatically induced errors and structural alterations of DNA are repaired by the cell. If there is no repair, DNA synthesis across the damaged site can lead to mutations, which may then lead to cancer or other diseases. Detection, identification, and quantification of mutations are important in understanding the mechanisms of mutagenesis and its role in disease and aging.

The bypass products from mutation profile studies, if viewed as oligonucleotides, are usually low in concentration, and most likely are mixtures of structural isomers. Our goal is to develop an on-line micro-HPLC electrospray tandem mass spectrometric method to separate, characterize, and quantify mixtures of isomeric oligonucleotide that can be isolated in mutation profile study.

All deoxyoligonucleotide samples used in this study were synthesized (on the 1- μ mol scale) by the Nucleic Acid Chemistry Laboratory at Washington University (St. Louis, MO) and were used without further purification. An HPLC system composed of two Ultra Plus micro-pumps and a controller (Micro-Tech; San Jose, CA, USA) was used as the solvent delivery system. 5 μ L of aqueous oligonucleotide solution (0.5 pmol/ μ L) was injected onto a 25 x 0.3 mm i.d. C18 reversed phase column. The samples were eluted from the column at 5 μ L/min using aqueous 0.4 M hexafluoro-2-propanol (HFIP) adjusted to pH 7.0 with triethylamine and a linear gradient of 20-60% methanol containing 0.4 M HFIP over 45 min [1,2].

ESI tandem mass spectrometry was performed on a Finnigan LCQ. The spray voltage was kept at 4.6 kV and the capillary temperature was 200°C. The capillary voltage was adjusted to -13.0 V for negative ion detection. In all experiments, helium was introduced to an estimated pressure of 1 mTorr for improving trapping efficiency. The background helium (2×10^{-5} Torr) served as collision gas during CAD. The collision energy was 40% of the maximum tickling voltage (5 V) for singly charged precursors, 20% for doubly charged precursors, and 15% for triply charged precursors.

Successful micro-HPLC separation and tandem mass spectrometric analysis were achieved for both T-rich and A-rich oligodeoxynucleotides. Solvent consumption (especially that of the expensive modifier, HFIP) is dramatically reduced than when using a regular HPLC pump followed by a 100:1 split.

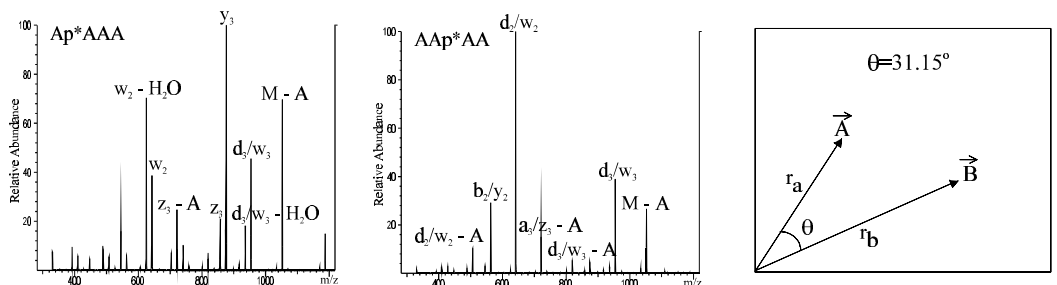
Figure 1 illustrates the vector representation for two isomeric modified oligonucleotides if the product-ion spectra are presented as vectors. Isomeric oligonucleotides can be quantitatively distinguished easily by the derived spectral contrast angle (θ). We found that this

method is more effective than using similarity indices [3], an approach we proposed some years ago.

In summary, multiple linear regression (MLR) using this vector representation and matrix notation was used to analyze multiple isomeric components that coeluted from HPLC to an ion trap mass spectrometer. This method can handle multiple components, use the whole mass spectrum to increase accuracy, and evaluate errors. A three-isomer mixture was analyzed by MLR, and the result is shown in Table 1. The success of the MLR method requires a well-behaved system (e.g., linear responses, no analyte-analyte interactions, and low noise). However, any irrelevant information (e.g., noise) can generate misleading calibration models and, therefore, unreliable MLR predictions. Before a principal component regression is developed, one should be careful to choose only relevant information (i.e., signature peaks of the isomers) based on the understanding of the fragmentation mechanism.

- 1: Alex Apffel and et al, *Anal. Chem.* **1997**, *69*, 1320-1325.
- 2: Richard H. Griffey and et al, *J. of Mass Specrom.* **1997**, *32*, 305-313.
- 3: Lay, J. O. and et al, *Org. Mass. Spectrom.* **1983**, *18*, 16.

Figure 1. Vector Representation



Compound A

Compound B

$$\cos \theta = \frac{\sum a_i b_i}{\sqrt{\sum a_i^2} \cdot \sqrt{\sum b_i^2}}$$

Table 1. MLR Result for a Three-Component Mixture Analysis

	three isomeric 5-mers mixed at 1 : 1 : 1 ratio		
MLR	Run # 1	Run # 2	Run # 3
measured ratio	1.00 : 1.15 : 1.05	1.00 : 1.15 : 1.08	1.00 : 1.14 : 1.06
esteemed error (%)	6.34 : 8.44 : 5.71	3.18 : 4.24 : 2.76	4.96 : 6.68 : 4.40

*This work was supported by NIH Center for Research Resources (Grant No. 2P41RR00954).