SHIMADZU

Development of a Modified, Compact Single Quadrupole Mass Spectrometer for Quality Control of Oligonucleotides

WP 342

Jeff Dahl[1]; Steve Kurzyniec[1]; Toshiya Matsubara[1]; Yoshio Takami[2]; So Kusumoto[2]; Ryo Kageyama[2]; Masaru Nishiguchi[2]; Kosuke Uchiyama[2]; and Atsuhiko Toyama[1]

[1]Shimadzu Scientific Instruments, Columbia, MD; [2]Shimadzu Corporation, Kyoto Japan

Introduction

Oligonucleotide drug products have become vital therapies in patient care. Production of purified, well-characterized oligos at scale requires laboratory analysis which can accurately detect and characterize a complicated array of closely-related impurities in the presence of the target product. We developed a modified single quadrupole mass spectrometer with an extended mass range and excellent high-mass sensitivity to improve analysis of oligonucleotides.

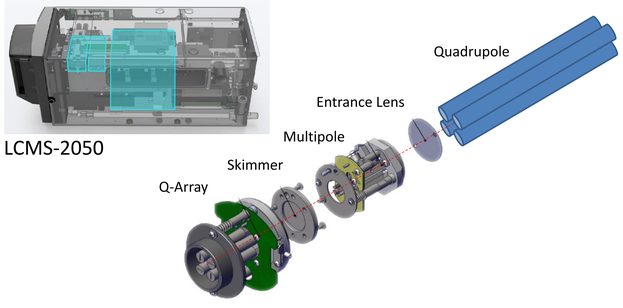

Figure 1. Illustration of the ion path of the modified LCMS-2050, highlighting parts that were changed in the new design.

Methods

A compact mass spectrometer (LCMS-2050, Shimadzu Corporation) was modified to extend the mass range and high m/z sensitivity. By changing the RF power supply, the mass range could be extended to *m/z* 3,000 without requiring an increase in RF or DC voltage, greatly simplifying the required design. The instrument was characterized and the resolution and mass axis was tuned manually using sodium iodide. Reference oligonucleotides were analyzed using a binary gradient LCMS system using ion pairing in negative ESI mode.

$$V = \frac{m\omega^2 r_0^2 \times q}{-4e}, U = \frac{m\omega^2 r_0^2 \times a}{-8e}$$

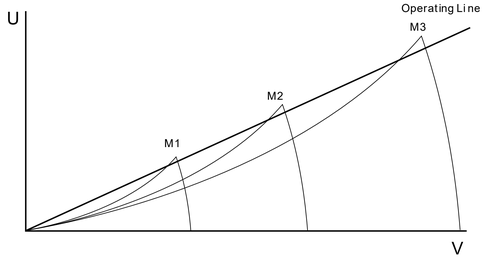

Figure 2. (Top) Equations for U and V showing their dependence on mass (m), angular frequency (ω), inscribed radius (r_0), elementary charge (e) and the Mathieu parameters (a, q). (Bottom) Plots of U and V voltage superimposed with the stability diagram of three different *m/z*.

Results

After installing the modified power supply, calibrant ions were introduced, and the RF gain, offset, and mass axis adjusted to produce mass spectra with appropriate resolution and *m/z*. The instrument was manually tuned in positive and negative mode by finding the optimum RF offset voltage and calibrated *m/z* value at the full range of scan speeds, and the result saved to the instrument's non-volatile memory.

Setting	Value
Pump A:	5 mM TBuAA, 1 uM EDTA, 10% acetonitrile in water
Pump B:	5 mM TBuAA, 1 uM EDTA, 80% acetonitrile in water
LC Flow Rate:	0.25 mL/min
LC Column:	Waters XBridge (3.5 µm, 130Å, 2.1 x 150 mm) C18 column
Oven Temp:	50 °C
MS Scan Range:	m/z 300 - 3000
Detector Voltage:	1.3 kV
Spray Voltage:	-3 kV
Nebulizer Gas:	2 L/min
Drying Gas:	5 L/min
Heating Gas:	7 L/min
Desolvation Temp:	500 °C
Desolvation Line Temp:	250 °C

Table 1. Method settings used for oligonucleotide analysis

Instrument performance was characterized by using standards of reserpine, calibrant ions, and test oligonucleotides.

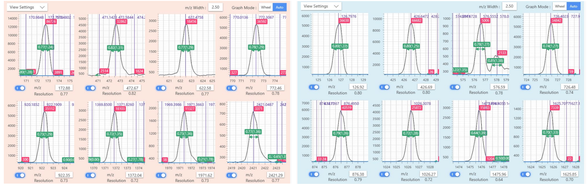

Figure 3. (Left) Result of manual tuning NaI ion clusers in positive mode. (Right) NaI clusters in negative mode. Unit mass resolution can be achieved throughout the mass range.

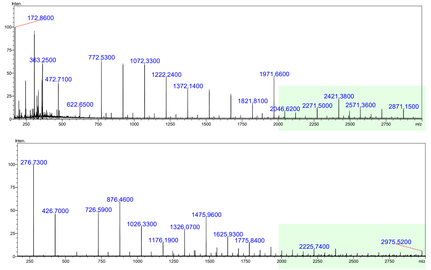

Figure 4. (Left) Full NaI spectrum in positive mode. (Bottom) full NaI spectrum in negative mode. Clusters can be detected up to *m/z* 3000 without losing signal intensity or resolution. Extended mass range shown in green shaded area.

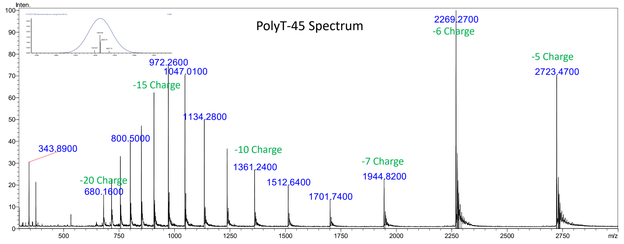

Figure 5. Mass spectrum of polyT-45 in negative mode. Ions of lower charge state can be detected at *m/z* greater than 3,000 improving deconvolution (inset).

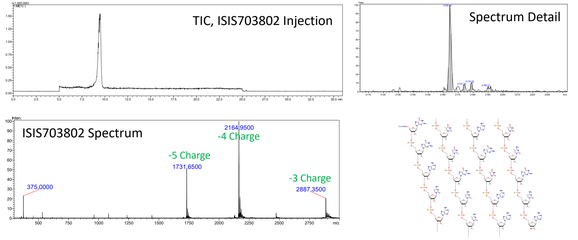

Figure 5. (Upper Left) TIC Chromatogram of the oligonucleotide ISIS703802. (Lower Left) Spectrum of ISIS703802 measured using the modified LCMS-2050. (Right) Zoomed spectrum of the -4 charge ion and structure of the oligo.

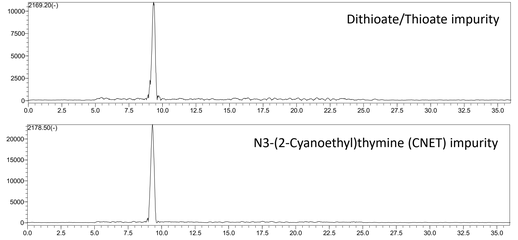

Figure 6. Chromatograms of two impurities detected in the analysis of ISIS703802.

Conclusion

We modified a compact LCMS-2050 single quadrupole mass spectrometer by extending the mass range to *m/z* 3000. We achieved satisfactory resolution and mass accuracy across the extended mass range and characterized oligonucleotides at higher *m/z*. Specific impurities were detected that can be recognized as common by-products. The instrument is well suited for efficient and effective quality control of oligonucleotides.