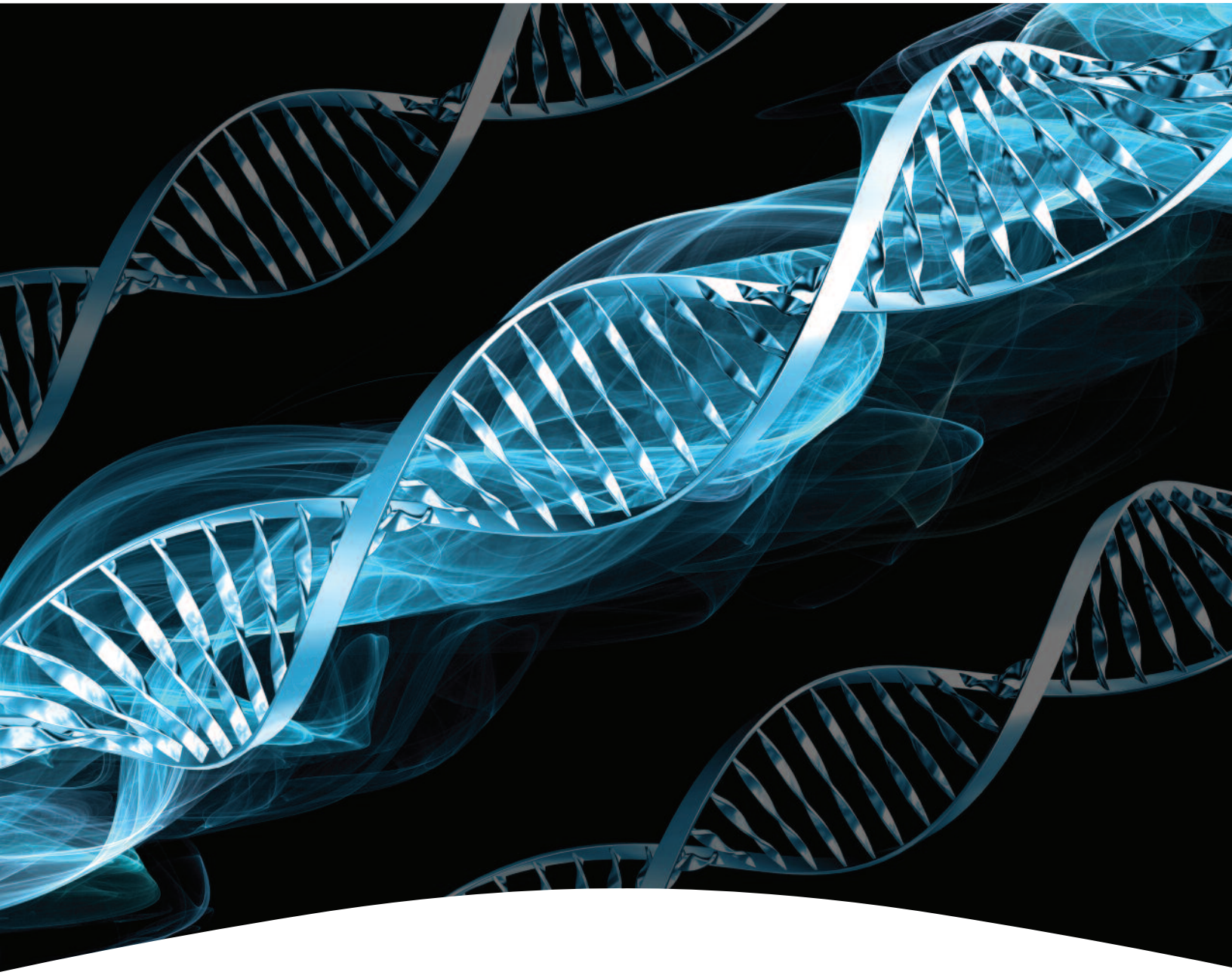
Honeywell Burdick & Jackson®



LC/MS Analysis of RNA Oligonucleotide Synthesized Utilizing rBMI Activator

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Since the introduction of our BioSyn® reagents for oligosynthesis over a decade ago, Honeywell Burdick & Jackson® has worked to understand the synthesis process and the role our reagents play in successful syntheses. Having invested in an ÄKTA™ 100, we utilize it for reagent quality testing and development work. We employ three methods for characterizing the oligonucleotide sequences that we synthesize: UV absorbance, HPLC and LC/MS. In this paper, we showcase the LC/MS data that was obtained during the development of our rBMI activator.

Honeywell Burdick & Jackson's patented* rBMI activator is a coupling reagent that is composed of 5-Benzylmercaptotetrazole (BMT) and N-Methylimidazole (NMI) in acetonitrile. Honeywell Burdick & Jackson formulates BMT with NMI and acetonitrile to increase the solubility of BMT in acetonitrile. We recommend using rBMI as an activator for RNA synthesis.

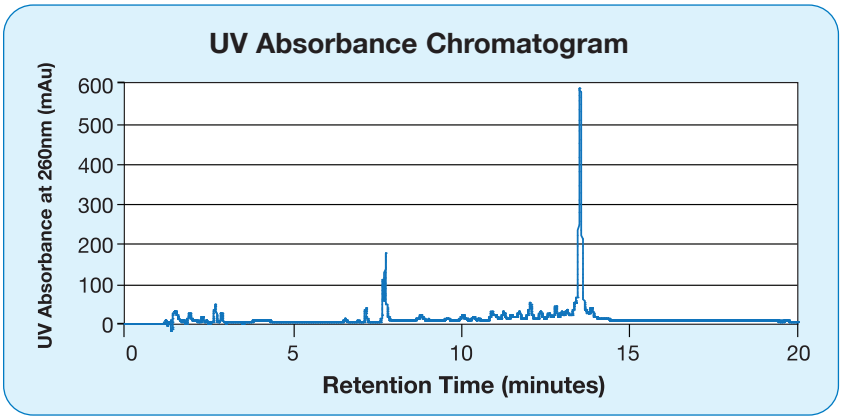

Figure 1

Identity and Relative Quantity of Selected Peaks From UV Absorbance Chromatogram

Peak #	Retention time (min.)	Identity from MS deconvolution	Area % using UV absorbance at 260nm
23	11.95	n-9	0.37
24	12.04	n-8	3.82
25	12.35	n-7	1.49
26	12.59	n-6	0.7
27	12.68	n-5	0.16
28	12.8	n-5	1.77
29	12.97	n-4	0.69
30	13.12	n-3	1.05
31	13.37	n-1, n-2	2.05
32	13.52	n	52.62
33	13.73	n	0.82
34	13.86	n + 24.53 Daltons	2.05

Table 1

LC/MS analysis is used to measure the purity of the synthesized RNA and identify the impurities that result from the synthesis process. As part of the development of our rBMI activator, we synthesized a 20-mer and confirmed that the oligosynthesis using rBMI and other B&J reagents results in RNA that consists primarily of the intended n-mers with minimal impurities (n+x -mers and impurity adducts).

Our LC/MS results for synthesis of a 20-mer (5' cga ucu ucu gga aau cca aT 3') are shown in *Figure 1*. The LC portion of the LC/MS instrument is utilized to separate the different n-mers from each other into peaks, as shown in the UV Absorbance Chromatogram. Integration of the UV absorbance at 260nm is used to determine the area percent of each of the n-mers as shown in *Table 1 (Note: The data shown is only a partial representation of peaks in the chromatogram).*

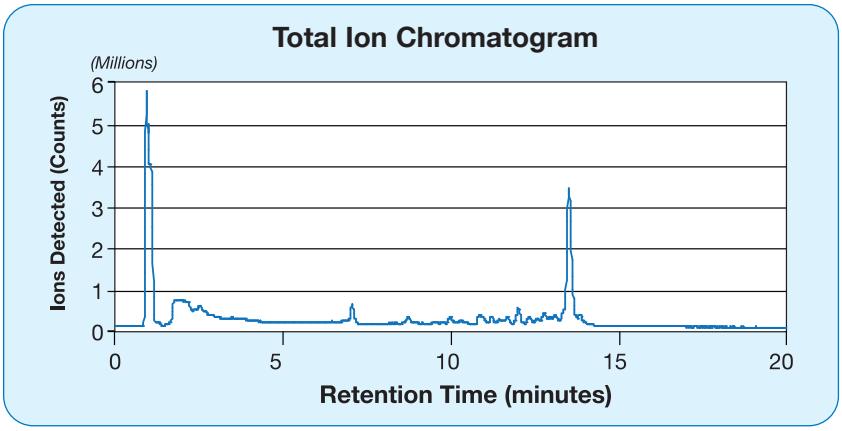

Figure 2

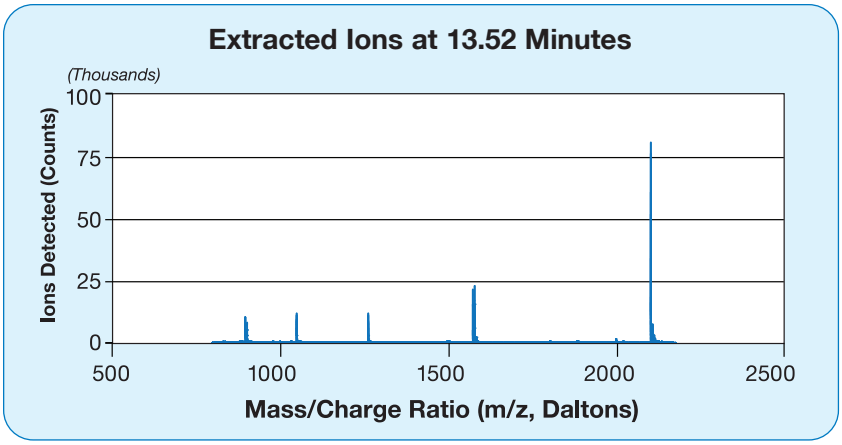

Figure 3 *Extracted Ion (m/z) Values: 900.45; 1,059.49; 1,260.99; 1,576.49; and 2,102.32*

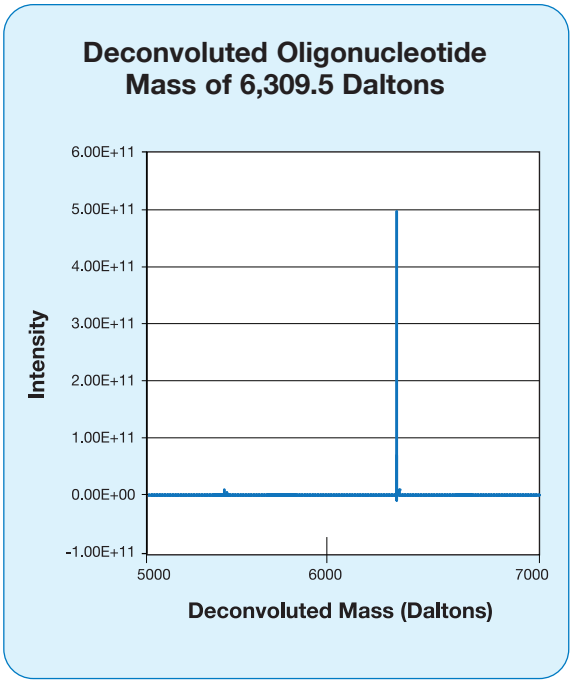

Figure 4 *ESI Scan (13.517 - 13.533 min, 2 scans) Deconvoluted (Isotope Width=1.0)*

The mass spectrophotometer is then used to determine the identity of the oligonucleotide corresponding to each peak. The instrument does not measure the mass of the oligonucleotide, only the mass/charge ratio (m/z). The instrument's detector dynamic range is from 50 to 3,200 Daltons. In this experiment, the detector range was set from 900 to 3,200 Daltons.

The total ion chromatogram *(see Figure 2)* is a measure of all the ions (m/z) that are detected for any particular retention time.

If we focus on the peak of interest, we can extract the m/z ratios for the ions that the mass spectrometer actually detected. These are shown for the peak at 13.52 minutes in the extracted ion chromatogram in *Figure 3*.

We can then use a computer algorithm to deconvolute the extracted ion m/z ratios into the "true" exact mass of the oligonucleotide at that retention time, as shown in *Figure 4,* and then compare that deconvoluted mass to the calculated mass/charge values for the RNA sequence *(see Table 2)*.

Based on a computed mass of 6,309.81 Daltons, our results indicate that 53.4% of the total synthesized RNA that was retained on the column is the desired product (n).

Calculated Mass/Charge Values for the RNA Sequence

RNA Sequence		5' cga ucu ucu gga aau cca aT 3'				
Oligonucleotide Species	exact mass (M in Daltons)	m/z for $(M-2H^+)^{2-}$	m/z for $(M-3H^+)^{3-}$	m/z for $(M-4H^+)^{4-}$	m/z for $(M-5H^+)^{5-}$	m/z for $(M-6H^+)^{6-}$
n	6309.81	3153.91	2102.27	1576.45	1260.96	1050.64
n-1 (c)	6004.63	3001.32	2000.54	1500.16	1199.93	999.77
n-2 (cg)	5659.42	2828.71	1885.47	1413.86	1130.88	942.24
n-3 (cga)	5330.22	2664.11	1775.74	1331.56	1065.04	887.37

Table 2

This experimental work is based on the following parameters:

- RNA sequence: 5’ cga ucu ucu gga aau cca aT 3’
- Synthesizer: ÄKTA™ oligopilot™ plus 100
- Synthesis parameters:
  - Primer support: GE Healthcare PS200 T80s (polystyrene beads, 80 µmole/g)
  - Amidites: 2’-tBDMS protected: A (n-bz), C (n-acetyl), G (n-acetyl), U
  - Synthesis scale: 100 µmole
- Activation parameters
  - Amidite excess: 1.6x
  - Coupling time: 3 minutes
- LC/MS parameters
  - LC/MS Instrument: Agilent 6210 Time-of-Flight (TOF) with Agilent Eclipse plus C18, 3.5 micron X 5 cm column
  - ESI Negative Mode
  - Buffer A: HFPIP and Diisopropylethylamine in water
  - Buffer B: Methanol
- Honeywell Burdick & Jackson BioSyn® reagents:
  - Deblock Reagent, cat. no. SR622 (3% Dichloroacetic acid in Dichloromethane (v/v))
  - Activator Reagent: rBMI Activator, cat. no. BR731RN (0.30M 5-Benzylthio-1H-tetrazole, 0.5% NMI, 99.5% Acetonitrile)
  - Capping Reagent Cap A**, cat. no. SR644 (20% Acetic Anhydride, 30% 2,6-Lutidine, 50% Acetonitrile (v/v/v))
  - Capping Reagent Cap B†, cat. no. BR654 (20% N-Methylimidazole, 80% Acetonitrile (v/v))
  - Oxidation Reagent, cat. No. BR664 (0.05M Iodine, 10% Water, 90% Pyridine (v/v))

Percent yield definitions:

LC/MS % FL (full length): The percent (as determined by the UV absorbance at 260nm) of the full-length oligomer with the correct mass as determined by deconvolution of the extracted ions at the retention time of the main peak.

For additional information or to request a sample, visit www.honeywell.com/contactbandj

* U.S. Patent No. 7,339,052
**GE Healthcare product designation for this formulation: Capping B
†GE Healthcare product designation for this formulation: Capping A

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