Analysis of Nucleosides Using an Agilent Infinity II High Speed UHPLC with the 6130 Single Quadrupole Mass Selective Detector

Application Note

Clinical Research

Author

Patrick Cronan

Agilent Technologies, Inc.

Lexington, Massachusetts, USA

Introduction

Nucleosides (adenosine, cytosine, guanosine, inosine, and uridine) are the structural subunits of nucleic acids. They are the biochemical precursors to nucleotides, and the building blocks of DNA and RNA. Nucleotides are essential to biological cell functions including data storage, energy production, cellular signaling, and coenzyme catalysis. As biological therapies are employed in our health care treatments, quick and easy detection of biological material, including nucleosides, becomes necessary.

LC/MS detection of nucleosides can be tricky since many molecules are positional isomers with the same molecular weight and the same *m/z* value. LC/MS methods tend to use long shallow gradients for accurate separation, as accurately separating the nucleosides on the UHPLC column is essential for correct mass detection.

An Agilent 1290 Infinity II high speed UHPLC system was used to develop a sub-10 minute LC method, achieving baseline resolution of 10 nucleosides without ion pair reagents. This, coupled to an Agilent 6100 Series Mass Selective Detector (MSD), enabled accurate characterization of all sample components with a limit of detection (LOD) of < 25 pg/µL.

Agilent Technologies

Materials and Methods

The Nucleosides Test Mix (p/n 47310-U) was purchased from Sigma-Aldrich. All chemicals and solvents were HPLC grade. Table 1 shows sample concentrations based on stock nucleoside concentration by dilution of the test mix to 1:10, 1:50, 1:100, 1:500, 1:1,000, 1:5,000, and 1:10,000.

The following configuration was used for the experiments:

- Agilent 1290 Infinity II Binary Pump with integrated vacuum degasser (G7120A)
- Agilent 1290 Infinity II Multisampler plumbed with ultra-low dispersion tubing kit (G7167A)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1290 Infinity II Diode Array Detector with 10 mm max light flow ultra-low dispersion (ULD) cell (G7117A)
- Agilent 6130 Single Quadrupole MSD with Jet Stream ESI source
- Agilent ZORBAX Eclipse Plus C18 column 2.1 × 100 mm, 1.8 µm

Instrument conditions

HPLC conditions

Instrument:	Agilent 1290 Infinity II Binary pump, multisampler, multicolumn thermostat, DAD
Column:	Agilent ZORBAX Eclipse Plus C18 2.1 × 100, 1.8 µm
Column temp.:	50 °C
Injection volume:	4 µL
Mobile phase:	A) Water 0.1% formic acid B) 80:20 Water:ACN – 0.1% formic acid
Flow rate:	0.1 to 0.8 mL/min

Gradient:

Time (min)	%A	%B	Flow (mL/min)
2.5	100.0	0	0.1
3.5	99.2	0.8	–
5.0	98.2	1.8	0.8
6.5	96.8	3.2	–
7.0	0	100	–
7.5	0	100	–
7.6	100	0	–

Stop time:	9 min
Post time:	0 min
Overall run time:	9.5 min

MS conditions

Instrument:	Agilent Jet Stream 6130 Mass Spectrometer
Ion mode:	AJS, ESI, positive
Gas temp.:	300 °C
Drying gas flow:	12 L/min
Nebulizer gas:	50 psi
Capillary voltage:	1,300 V
Nozzle voltage:	2,000 V
Sheath gas flow:	11 L/min
Sheath gas temp.:	250 °C

Table 1. Nucleoside dilution series concentration.

	Thiocytidine	2-O-Methylcytidine	Guanosine, Inosine, 1-Methyladenosine, 7-Methylguanosine, Pseudouridine, Uridine	Cytidine, Ribothymidine	5-Methylcytidine, 3-Methylcytidine
Stock standard	**10,000 µg/µL**	**20,000 µg/µL**	**25,000 µg/µL**	**50,000 µg/µL**	**100,000 pg/µL**
10x	1,000 pg/µL	2,000 pg/µL	2,500 pg/µL	5,000 pg/µL	10,000 pg/µL
50x	200 pg	400 pg	500 pg	1,000 pg	2,000 pg
100x	100 pg	200 pg	250 pg	500 pg	1,000 pg
500x	20 pg	40 pg	50 pg	100 pg	200 pg
1,000x	10 pg	20 pg	25 pg	50 pg	100 pg
5,000x	2 pg	4 pg	5 pg	10 pg	20 pg
10,000x	1 pg	2 pg	2.5 pg	5 pg	10 pg

Results and Discussion

The goal of this separation was chromatographic resolution to enable accurate LODs and limits of quantitation (LOQs) of 12 nucleosides in under 10 minutes (Figure 2). After the dilution series was analyzed in triplicate, linear regression lines were plotted. Figure 1 shows the most and least abundant nucleoside. The linear regression plot shows good correlation over four orders of magnitude, with R^2 values of 0.999 at the LOD of each nucleoside.

All 12 nucleosides can be detected with extracted ion chromatograms from the Agilent 6130 Single Quadrupole MSD (Figure 2). Ten of 12 nucleosides are chromatographically resolved, demonstrating the advantage of mass spectrometry for difficult-to-resolve compounds.

The LODs for 12 nucleosides ranged from < 4–50 pg/µL, and the LOQs ranged from <10–250 pg/µL. All statistics can be found in Table 2. The retention time reproducibility for all 21 injections was < 0.3% RSD (Table 3).

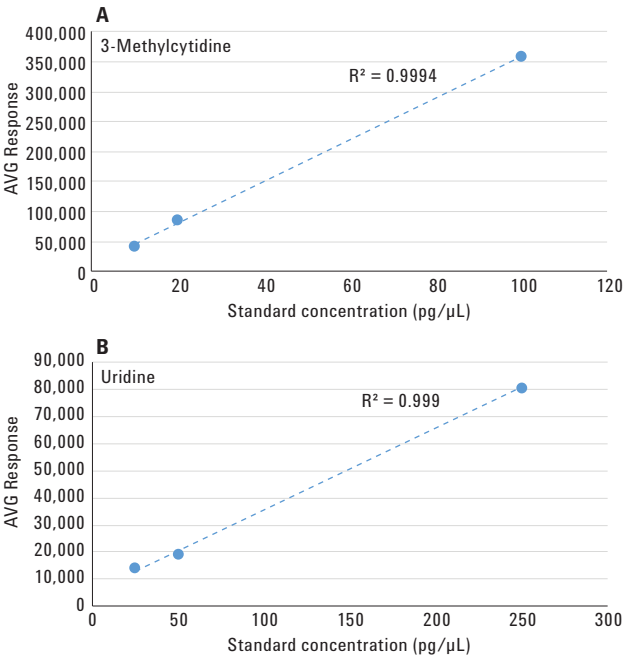

Figure 1. Linear Regression lines for an abundant (3-Methylcytidine, A) and least abundant (Uridine, B) nucleoside at LOD/LOQ.

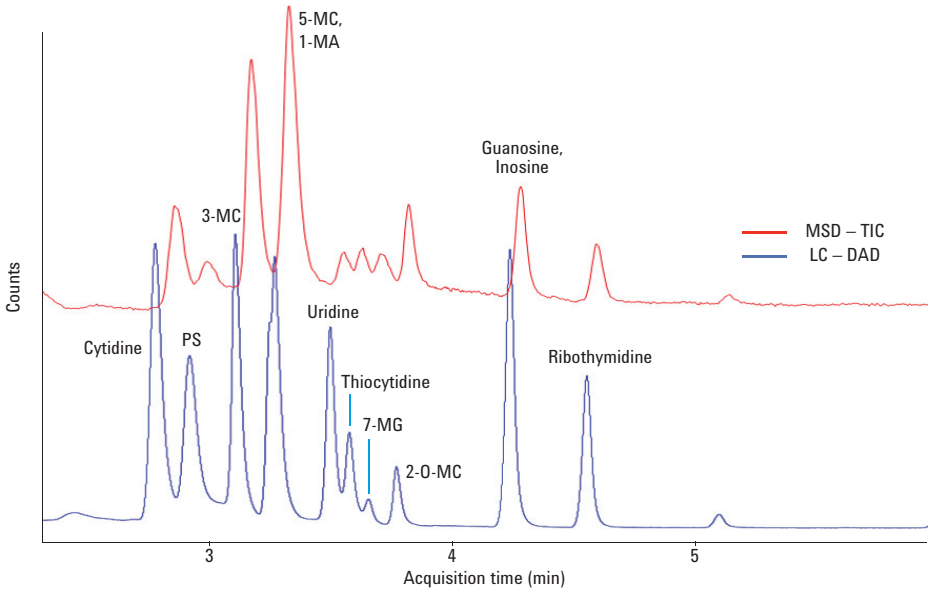

Figure 2. Red: Total ion chromatogram from MS showing the separation of 12 nucleosides. Blue: Diode array detector signal of the chromatographic separation of 10 of the 12 nucleosides in the standard.

Table 2. LOD and LOQ calculations for all 12 nucleosides present in the standard text mix.

Peak 1	Nucleoside	S/N	LOD (pg/µL)	S/N	LOQ (pg/µL)	Formula	MI Mass	*m/z* (1+)
1	Cytidine	5.8	<5	10	<10	$C_9H_{13}N_3O_5$	243.08	244.1
2	Pseudouridine	4.3	<25	26.9	<250	$C_9H_{12}N_2O_6$	244.07	245.2
3	3-methylcytidine	8.3	<10	17.7	<20	$C_{10}H_{15}N_3O_5$	257.1	258.2
4	5-methylcytidine	9.2	<10	22.3	<20	$C_{10}H_{15}N_3O_5$	257.1	258.2
5	1-methyladenosine	5.0	<5	25.1	<25	$C_{11}H_{15}N_5O_4$	281.11	282.2
6	Uridine	3.2	<25	24.1	<250	$C_9H_{12}N_2O_6$	244.07	245.2
7	Thiocytidine	7.6	<20	10.2	20	$C_9H_{13}N_3O_4S$	259.06	260.1
8	7-methylguanosine	4.6	<25	45.3	<250	$C_{11}H_{15}N_5O_5$	297.11	298.2
9	2-O-methylcytidine	4.4	<4	22.2	<20	$C_{10}H_{15}N_3O_5$	257.1	258.2
10	Inosine	8.7	<25	14.1	<50	$C_{10}H_{12}N_5O_5$	268.08	269.2
11	Guanosine	7.6	<25	12.2	25	$C_{10}H_{13}N_5O_5$	283.09	284.2
12	Ribothymidine	6.1	<50	11.4	<100	$C_{10}H_{14}N_2O_6$	258.09	259.1

Table 3. Excellent percent RSDs for the standard test mix on the Agilent 1290 Infinity II demonstrating overall system reproducibility. All values are less than 0.3% RSD.

	Cytidine RT (min)	PS RT (min)	3-MC RT (min)	1-MA RT (min)	5-MC RT (min)	Uridine RT (min)	Thiocytidine RT (min)	7-MG RT (min)	2-O-MC RT (min)	Guanosine RT (min)	Inosine RT (min)	RBT RT (min)
Mean	2.82	2.96	3.14	3.27	3.30	3.52	3.60	3.67	3.79	4.25	4.25	4.56
Std dev	0.0069	0.0085	0.0036	0.0057	0.0031	0.0031	0.0028	0.0044	0.0023	0.0025	0.0025	0.0038
%RSD	0.246	0.289	0.114	0.173	0.093	0.087	0.080	0.121	0.062	0.058	0.058	0.082

PS = Pseudouridine, 3-MC = 3-Methylcytidine, 1-MA = 1-Methyladenosine, 5-MC = 5-Methylcytidine, 7-MG = 7-Methylguanosine, 2-O-MC = 2-O-Methylcytidine, RBT = Ribothymidine

Conclusions

The Agilent 1290 Infinity II UHPLC system coupled with the Agilent 6130B Single Quadrupole MSD provides an optimal solution to attain low limits of detection for closely eluting nucleosides. The low delay volume and active dampening of the Agilent 1290 Infinity high speed pump enables better resolution with shorter analysis times, leading to improved mass spectral sensitivity.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures.

This information is subject to change without notice.

Printed in the USA
May 5, 2016
5991-6871EN

Agilent Technologies