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Analysis of Elemental Impurities in Synthetic Oligonucleotides by ICP-MS In compliance with USP <232>/<233> and ICH Q3D(R2)/Q2(R1)

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Introduction

Synthetic oligonucleotides are short chains (oligomers) of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules. They are used in applications such as polymerase chain reaction (PCR) tests, DNA sequencing and novel therapeutic products.

The synthesis of oligonucleotides involves a four-step cyclic process followed by multi-step downstream purification. This manufacturing process as well as environment, packaging, and container closure systems can be potential sources of contamination leading to inorganic impurities formation.

To control and monitor product safety, permitted daily exposure (PDE) limits of 24 elements are defined in ICH guideline Q3D(R2)[1] and USP chapter <232>[2]. ICH Q2(R1)[3] and USP<233> [4] describe analytical measurement requirements and procedures for the measurement of those elemental impurities. Although oligonucleotide products have not been included in those regulatory guidelines yet, the industry common practice is to follow those guidelines.

In this study, an analytical workflow (Figure1) was developed in accordance with the ICH/USP guidelines for the analysis of elemental impurities in an oligonucleotide sample using an Agilent 7850 ICP-MS. Data was acquired to evaluate the accuracy, specificity, reproducibility, and ruggedness of the method.

Experimental

Sample preparation

The oligonucleotide sample was at a concentration of 10 mg/mL. A 0.1 g aliquot of the sample was diluted 500 times by diluent containing 3% HNO_3 and 1% HCl.

Instrumentation

A preset ICH/USP method was selected from the ICP-MS MassHunter software. The preset method automatically sets all the major instrument operation parameters (Table 1), loads the targeted elements' list, and lists QC limits based on parenteral, oral, and inhalation PDEs.

Parameters	Settings
RF power (W)	1550
Spray Chamber Temp (˚C)	2
Sampling Depth (mm)	10
Nebulizer Gas Flow (L/min)	0.85
Make-up gas (L/min)	0.2
Extract 1 (V)	–9.3
Extract 2 (V)	–200
Omega Bias (V)	–70
Omega Lens (V)	6.8
Deflect (V)	1.8
Cell Mode	Helium
He Gas Flow (mL/min)	4.5
KED (V)	5.0

Table 1. Agilent 7850 ICP-MS operating parameters.

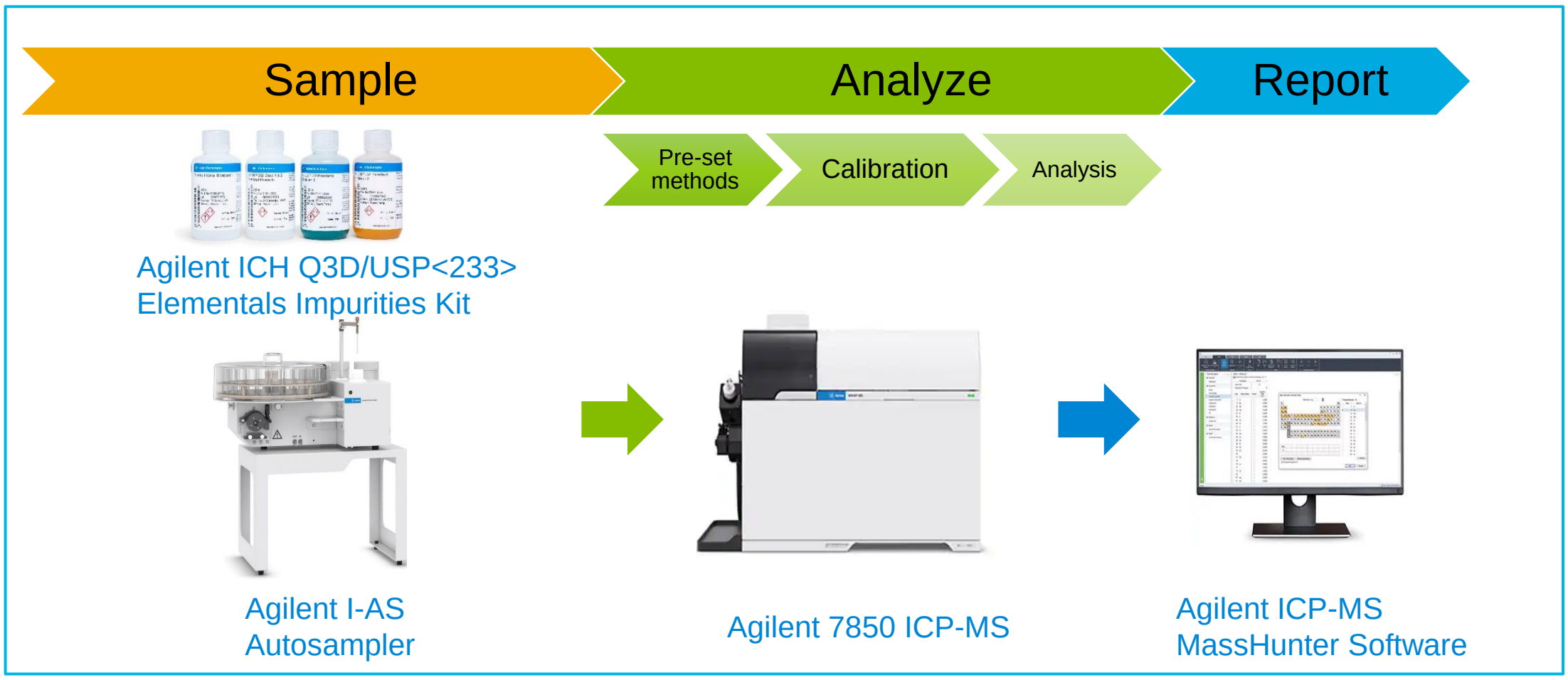

Figure 1. ICP-MS workflow for elemental impurity analysis in oligonucleotide.

Results and Discussion

J value

The "J-value" is the Target Concentration value in the prepared sample, which defines the maximum permitted concentration limit for the analyte in the sample. J value is calculated from:

$$J = \frac{PDE}{Total\ Dilution \times Max\ Daily\ Dose}$$

In this study, the J value is calculated based on a 1 g/day maximum dose and sample dilution factor of 500.

Drift and QC stability

Drift results of 1.5J standard before (n=3) and after sample (n=3) were well within ±20% limit as required by USP<233>. QC measurement (n=16) was consistent throughout 7 hours of batch with RSD less than 2.6% for all elements. Both results demonstrated system suitability of the 7850 ICP-MS method for the routine analysis of oligonucleotide samples.

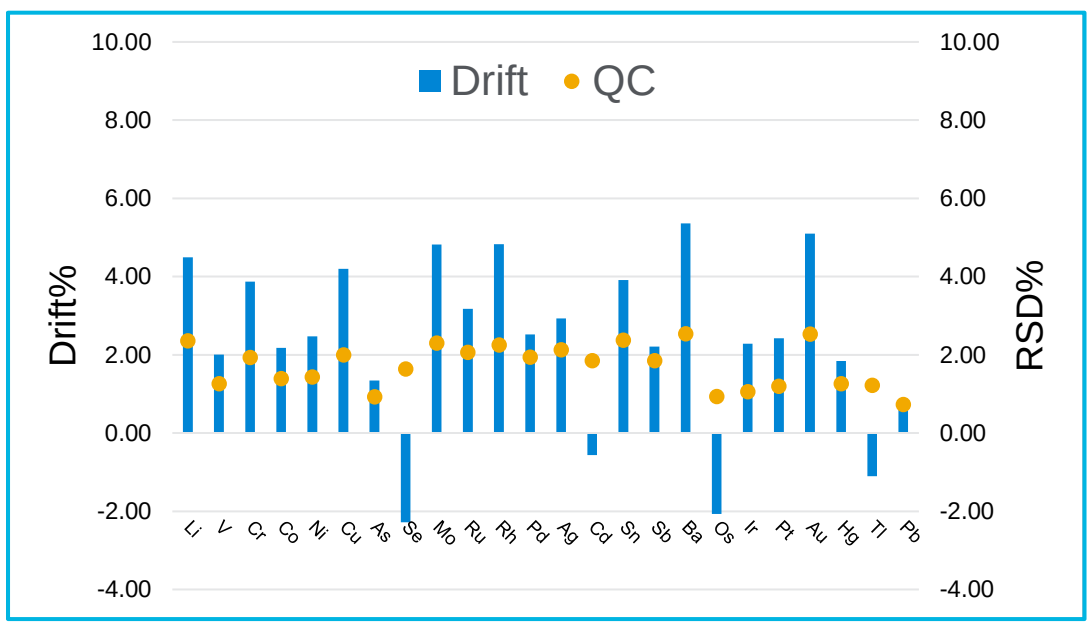

Figure 2. System suitability of drift and QC stability.

Detectability

The recovery of three independently prepared 1 J spiked samples was within 85-115% of the measured concentration of the 1 J standard as listed in Table 2. Also, the mean concentration of the 0.8 J spiked samples (n=3) was lower than the measured concentration of the 1 J standard. Both results passed USP <233> acceptance criteria.

Precision

The precision of six independently prepared 1 J spiked samples (n=6) was less than 2.5% as shown in the second last column in Table 2. It was well within the acceptance criteria of NMT 20%.

Specificity

Helium cell gas and KED removes the polyatomic ion[5] and results of secondary isotopes acquired were in good agreement with their primary isotopes for all the elements.

Ruggedness

The results of the intermediate precision test of 12 independent 1 J spiked samples run on a different day and by a different operator are shown in last column of Table 2. The intermediate precision was less than 4% RSD.

Accuracy

The spike recovery results for 0.5 J, 1 J and 1.5 J spiking levels in three independent sample preparations were all between 85 to 115% (Figure 3), meeting the performance criteria.

For all elements, excellent linearity was achieved with linear regression values > 0.999. Low background equivalent concentrations (BECs) and LOQs were all at the ppt level.

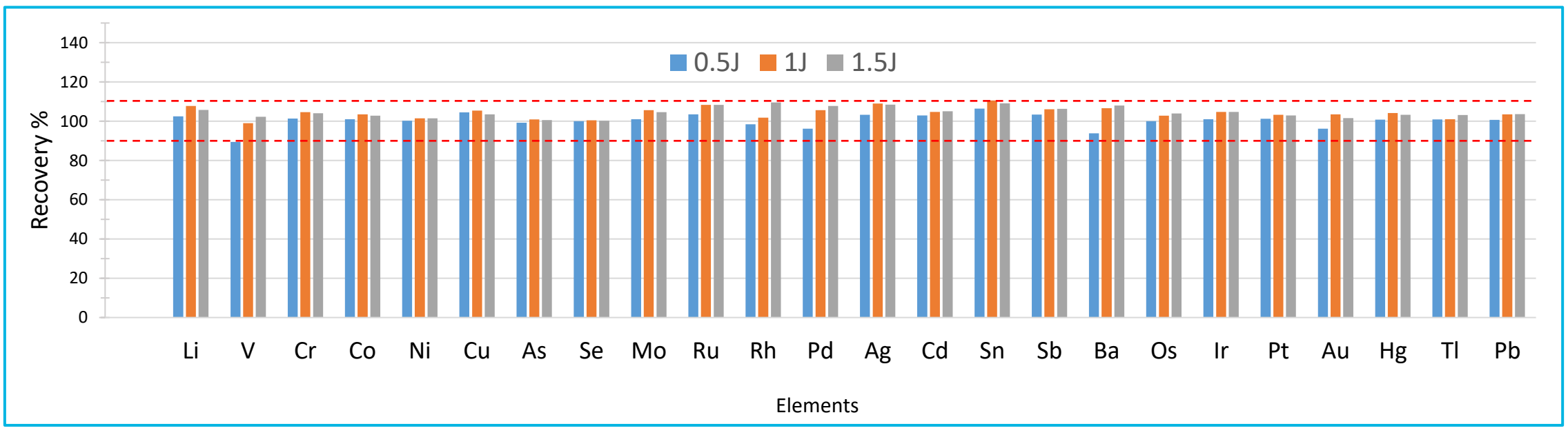

Figure 3. Accuracy results of spiked samples at 0.5 J, 1 J , and 1.5 J concentrations.

Results and Discussion

Table 2. Results of detectability, precision , and ruggedness of regulated elements.

Mass	Element	Actual 1 J Values (µg/L)	Measured		Recovery (%)	0.8 J Spike (µg/L)	0.8 J Spike/1 J Standard (%)	1 J Spike %RSD (n=6)	1 J Spike %RSD (n=12)
			1 J Standard (µg/L)	1 J Spike (µg/L)					
7	Li	500	503	542	107	414	82.3	1.2	3.3
51	V	20	20.3	20.3	100	15.3	75.1	1.0	2.8
52	Cr	2200	2223	2326	105	1794	80.7	1.1	4.1
59	Co	10	10.1	10.4	104	8.09	80.4	1.0	3.5
60	Ni	40	40.2	41.0	102	32.3	79.8	1.1	3.6
63	Cu	600	602	636	105	500	83.0	1.0	4.4
75	As	30	30.1	30.3	101	24.1	80.1	1.3	3.2
78	Se	160	160	161	100	128	79.9	1.6	4.0
95	Mo	3000	3034	3207	106	2457	80.9	1.0	4.1
101	Ru	20	20.1	21.8	108	16.1	80.0	2.0	3.8
103	Rh	20	20.1	21.1	105	15.3	76.1	2.0	4.3
105	Pd	20	20.2	21.8	108	15.4	76.6	2.1	3.6
107	Ag	20	20.2	22.0	109	16.2	80.1	2.0	3.8
111	Cd	4	4.03	4.33	108	3.19	79.3	2.5	3.7
118	Sn	1200	1208	1367	113	994	82.1	2.0	3.9
121	Sb	180	181	195	108	145	79.9	2.1	4.3
137	Ba	1400	1434	1540	107	1140	79.5	2.2	3.7
189	Os	20	20.0	20.6	103	15.9	79.5	1.0	1.8
193	Ir	20	20.0	20.9	105	16.2	81.0	0.9	2.8
195	Pt	20	20.0	20.7	103	16.3	81.3	0.9	3.1
197	Au	200	201	210	105	161	79.8	1.4	4.0
201	Hg	6	5.98	6.23	104	4.87	81.5	1.2	3.2
205	Tl	16	16.0	16.2	101	12.8	79.7	1.1	1.9
208	Pb	10	10.0	10.4	104	8.03	80.1	0.8	2.7

Conclusions

The study has demonstrated the suitability of the Agilent 7850 ICP-MS for the identification and quantification of 24 elements in a synthetic oligonucleotide sample in compliance with existing USP and ICH guidelines. Instrument and method performance requirements for stability, detectability, precision, specificity, ruggedness, accuracy, and spike recovery were all achieved per the criteria given in USP <232>/<233> and ICH Q3D(R2)/Q2(R1).

As the development and use of oligonucleotides in therapeutic applications continue to advance, the elemental impurities analysis of oligonucleotides using 7850 ICP-MS can safeguard the product quality and safety.

References

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