Application Note

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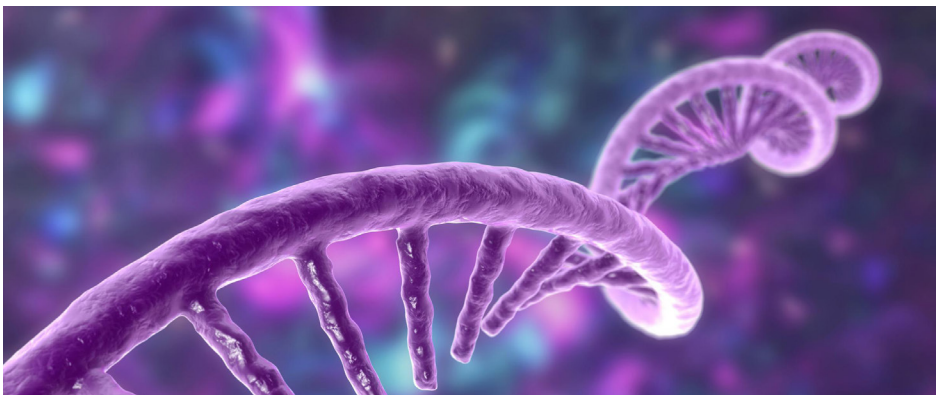
Analysis of Elemental Impurities in Synthetic Oligonucleotides by ICP-MS

Development and testing of an efficient Agilent 7850 ICP-MS method 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, synthesized with a specific sequence of nucleotides. Oligonucleotides are used in applications such as polymerase chain reaction (PCR) tests and DNA sequencing. In the biopharmaceutical industry, therapeutic oligonucleotides with novel modality are enabling a revolutionary approach to treating conditions including cancer, neurological, metabolic, musculoskeletal, sensory, and cardiovascular diseases. The biopharma sector is growing due to expedited and flexible regulatory approvals in many jurisdictions that have allowed for faster commercialization of oligonucleotide-based therapeutics. To date, 16 synthetic oligonucleotide therapeutics have been approved by various regulatory authorities.

The synthesis of most oligonucleotides is based on phosphoramidite chemistry and involves a four-step cyclic process on a solid phase resin (*1*). Following the synthesis, the oligonucleotides undergo downstream processing steps including chromatographic purification, desalting, and drug substance isolation. The process, which is outlined in Figure 1, involves the use of various chemicals and solvents that could be a potential source of contamination of the final therapeutic, including organic and inorganic (elemental) impurities. The manufacturing environment, packaging, and container closure systems (CCS) are other potential sources of contamination. The presence of impurities in a drug product can lead to a shorter shelf-life or unexpected side-effects and some contaminants, such as heavy metals, are inherently harmful.

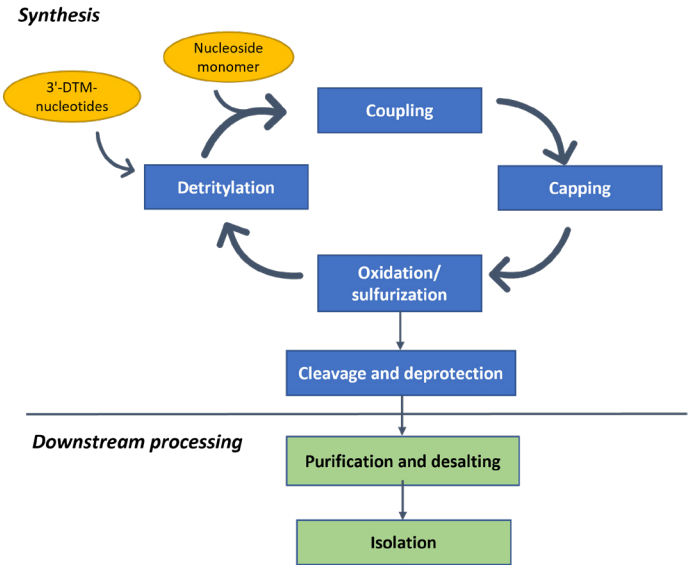

Figure 1. The oligonucleotide synthesis cycle and downstream processing steps.

To control and monitor drug safety, national and regional regulatory bodies publish regulations that specify impurity limits. The most widely accepted and referenced standards on elemental impurities are defined in ICH guideline Q3D(R2) (*2*) and USP National Formulary (NF) chapter <232> Limits (*3*). These chapters include an extensive list of elemental impurities that must be monitored and controlled in drugs.

The maximum exposure limit or permitted daily exposure (PDE) for an element is defined according to its toxicity and route of administration. ICH Q2(R1) (*4*) and USP<233> Procedures (*5*) describe analytical measurement requirements and procedures based on the use of ICP-OES or ICP-MS technologies for the measurement of elemental impurities.

Since only a limited number of therapeutic oligonucleotides have been commercialized so far, there are currently no specific ICH, USP, or FDA regulatory guidelines or quality standards for oligonucleotide products. However, it is common practice for analysts to apply the ICH Q3D (R2) guidelines for the determination of elemental impurities in oligonucleotide products (*6*).

In this study, an analytical method 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 for the measurement of elemental impurities in a synthetic oligonucleotide.

Experimental

System suitability tests

The sample preparation and method validation procedures defined in USP <233> were used for the system suitability testing of the 7850 ICP-MS method. The sample was a synthetic antisense oligonucleotide, 21 nucleotides in length. Because most oligonucleotide therapeutics are administrated through intravenous injection, the parenteral PDE limits for elemental impurities in drug products were chosen as reference limits. Parenteral PDE limits are much lower than the limits of other administration routes, such as oral and cutaneous.

For each sample, mixed standards containing all the regulated elements were prepared to give appropriate spike concentrations (0.5, 0.8, 1.0, and 1.5 J) based on the parenteral limits. 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 (*7*). J values are calculated from:

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

A 1.5 J standard was used as both a drift and stability QC check for the system suitability test of stability.

The J-value is also used to define the calibration levels and QC concentrations. For example, calibrations must be prepared at concentration levels between 0.5 and 1.5 J. Detectability (for Limit procedures) must be demonstrated using a sample spiked at 80% of the J value (0.8 J). Spike recovery tests must also be performed at concentrations ranging from 50 to 150% of the J value (i.e., between 0.5 and 1.5 J).

Sample preparation

The synthetic antisense oligonucleotide sample was provided in liquid form at a concentration of 10 mg/mL. A 0.1 g aliquot of the sample was solubilized at room temperature in 30 mL of a diluent solution containing 3% HNO_3 and 1% HCl in ultrapure water. The solution was made up to a final weight of 50 g by adding more of the diluent solution to give a total dilution factor of 500 times.

Instrumentation

The elemental impurities defined in the ICH/USP methods were measured using an Agilent 7850 ICP-MS operated using Agilent ICP-MS MassHunter software version 5.2. The 7850 includes the ORS^4 collision cell and the instrument was fitted with the standard sample introduction system, comprising a MicroMist glass concentric nebulizer, quartz spray chamber, and quartz torch with 2.5 mm injector, and nickel sampling and skimmer cones. An 89-rack Agilent I-AS autosampler was used to introduce the samples to the ICP-MS.

A preset ICH/USP method was selected from the ICP-MS MassHunter software. The preset method automatically sets all the major instrument operation parameters, loads the targeted elements' list, and lists QC limits based on parenteral, oral, and inhalation PDEs, per the workflow shown in Figure 2.

Most of the parameters in Table 1 were set automatically by the software. The nebulizer and make-up gas flow rates were adjusted to maximize sensitivity. Operating the ORS^4 in helium (He) mode is the standard method used on Agilent ICP-MS systems, as it can reliably remove the typical polyatomic ion interferences on all common analytes using kinetic energy discrimination (KED) (*8*). The lens voltages were autotuned one time only and the same tune conditions were used for all elements. The preset method and autotuning function simplified the method development process, which is an important consideration in labs that may be new to inorganic mass spectrometry instrumentation and operation.

Table 1. Agilent 7850 ICP-MS operating conditions.

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

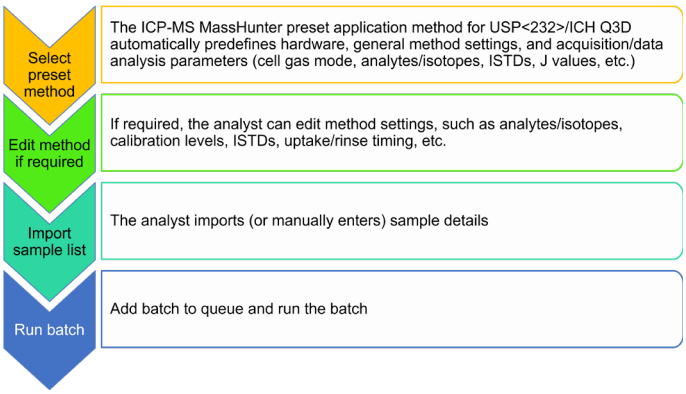

Figure 2. Method setup using the Agilent ICP-MS MassHunter Preset Method for ICH/USP elemental impurities.

Table 2 shows the predefined preferred measurement mass (isotope) for each regulated element and the parenteral PDE for each element. The J value in the table is calculated based on a 1 g/day maximum dose and sample preparation dilution factor of 500 (0.1 g in 50 g). In this study, the J values, which are equivalent to the PDEs after correction for the sample preparation dilution factor, are the actual elemental concentrations measured in the sample solutions during the ICP-MS analysis.

Table 2. List of regulated elements, primary isotopes, parenteral dosage PDE, and J-value based on 500x dilution and maximum daily dose of 1 g/day.

Mass	Element	Parenteral Dosage PDEs (µg/day)	J Values (µg/L, ppb) at 500x Dilution; 1 g/day max dose
ICH/USP Class 1			
111	Cd	2	4
208	Pb	5	10
75	As	15	30
201	Hg	3	6
ICH/USP Class 2A			
59	Co	5	10
51	V	10	20
60	Ni	20	40
ICH/USP Class 2B			
205	Tl	8	16
197	Au	100 (300*)	200
105	Pd	10	20
193	Ir	10	20
189	Os	10	20
103	Rh	10	20
101	Ru	10	20
78	Se	80	160
107	Ag	10 (15*)	20
195	Pt	10	20
ICH/USP Class 3			
7	Li	250	500
121	Sb	90	180
137	Ba	700	1400
95	Mo	1500	3000
63	Cu	300	600
118	Sn	600	1200
52	Cr	1100	2200

**PDEs for Ag and Au specified in ICH Q3D(R2).*

Results and discussion

Before being used for limit or quantitative procedures, analytical instruments and methods must meet performance criteria that are defined in detail in ICH Q2(R1) and USP<233>. According to USP<233>, system suitability must be demonstrated using a drift check, with performance data falling within the acceptable range criteria. Limit procedures check detectability, precision, and specificity while quantitative procedures must demonstrate acceptable performance for accuracy, precision (both repeatability and ruggedness), specificity, limit of quantitation, range, and linearity.

Drift check and QC stability check

Long-term stability is an important system suitability test. To check for signal drift, a 1.5 J standard was analyzed using the 7850 ICP-MS before and after sample analysis. The drift results for all elements were well within the ±20% limit, as shown in Table 3, with most elements showing drift below 5%.

The 1.5 J standard also served as a QC check. The QC solution was repeatedly analyzed every 15 samples throughout the whole batch run that lasted over 7 hours. The relative standard deviation (RSD) of the 16 measurements of each element was less than 2.6% in all cases and below 1% for several elements including the critical Class 1 contaminants, As and Pb. Both the drift and QC check results confirmed that the initial calibration remained valid throughout the course of the batch. The drift check results also demonstrated the stability and robustness of the 7850 ICP-MS for the routine analysis of oligonucleotide samples.

Table 3. Drift check results for the 1.5 J standard before and after the sample batch run and QC stability results of the 1.5 J standard throughout the batch run of 7 hours (n=16).

Mass	Element	Parenteral Dosage PDEs (µg/day)	J Values (µg/L, ppb) at 500x Dilution; 1 g/day max dose	1.5 J Actual Values (µg/L)	1.5 J Measured Result (µg/L)		Drift%	%RSD (n=16)
					Before Sample (n=3)	After Sample (n=3)		
7	Li	250	500	750	756	790	4.5	2.4
51	V	10	20	30	30.7	31.4	2.0	1.3
52	Cr	1100	2200	3300	3320	3450	3.9	1.9
59	Co	5	10	15	15.1	15.4	2.2	1.4
60	Ni	20	40	60	60.0	61.5	2.5	1.4
63	Cu	300	600	900	908	946	4.2	2.0
75	As	15	30	45	45.2	45.8	1.3	0.9
78	Se	80	160	240	245	240	−2.3	1.6
95	Mo	1500	3000	4500	4540	4780	4.8	2.3
101	Ru	10	20	30	30.3	31.3	3.2	2.1
103	Rh	10	20	30	31.3	32.7	4.8	2.3
105	Pd	10	20	30	30.3	31.1	2.5	1.9
107	Ag	10	20	30	30.2	31.1	2.9	2.1
111	Cd	2	4	6	6.09	6.06	−0.6	1.9
118	Sn	600	1200	1800	1810	1880	3.9	2.4
121	Sb	90	180	270	272	278	2.2	1.9
137	Ba	700	1400	2100	2130	2220	5.4	2.5
189	Os	10	20	30	30.5	29.8	−2.1	0.9
193	Ir	10	20	30	30.1	30.8	2.3	1.1
195	Pt	10	20	30	30.2	30.9	2.4	1.2
197	Au	100	200	300	304	319	5.1	2.5
201	Hg	3	6	9	9.06	9.23	1.8	1.3
205	Tl	8	16	24	24.1	23.8	−1.1	1.2
208	Pb	5	10	15	15.1	15.2	0.6	0.7

Limit procedures

Detectability

According to USP<233>, detectability is demonstrated by measuring samples spiked at 1 and 0.8 J and comparing the results to a 1 J standard. The mean concentration of three independently prepared 1 J spiked samples (n=3) must be within ±15% of the measured concentration of the 1 J standard. Also, the mean concentration of the 0.8 J spiked samples (n=3) must be lower than the measured concentration of the 1 J standard. The detectability results of this study, which are shown in Table 4, passed both acceptance criteria.

Precision (repeatability)

USP<233> acceptance criteria for precision state that the RSD for the measurement of six independently prepared 1 J spiked samples (n=6) must not be more than 20%. The precision of the 7850 ICP-MS measurements was less than 2.5%, well within the acceptance criteria (Table 4).

Specificity

According to ICH Q2(R1), the specificity test demonstrates that the analytical procedure can unequivocally assess the target element in the presence of the sample matrix and other analytes. ICP-MS is intrinsically specific as it detects each target element based on its unique isotopic mass. Commonly occurring polyatomic ion interferences are removed by the ORS^4 collision cell using He cell gas and KED. He-KED selectively removes the polyatomic ions (which have a larger ionic cross section) from the ion beam, allowing the analyte ions to be measured free from spectral overlaps (*8*). He KED is a universal cell mode (same conditions used for all analytes), so the standard measurement conditions also give access to secondary, or "qualifier" isotopes for most elements. The qualifier isotopes were included in the acquisition and results showed good agreement with the preferred isotope results, confirming the measurement at the primary isotopes (data not shown).

Table 4. Results of detectability, precision (repeatability), and intermediate precision (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

Quantitative procedures

Accuracy

The accuracy of the quantitative procedure must be demonstrated by measuring spiked samples at concentrations of 0.5, 1, and 1.5 J in three independent samples. Spike recoveries must be within 70 to 150% at each concentration, after subtraction of the concentration of the element in the unspiked sample. As shown in Figure 3, the spike recovery results for the 7850 ICP-MS at each spike level were all between 85 to 115%, easily meeting the performance criteria.

The accuracy test can also be used to demonstrate performance for limits of quantification (LOQs), analytical range, and linearity. Figure 4 displays exemplary calibration curves of some elements including the Class 1 elements (As, Cd, Hg, and Pb), two low-level Class 2A elements (Co and Ni), and Pd and Pt. For all elements, excellent linearity was achieved with linear regression values > 0.999. Background equivalent concentrations (BECs) and LOQs were all at the ppt level.

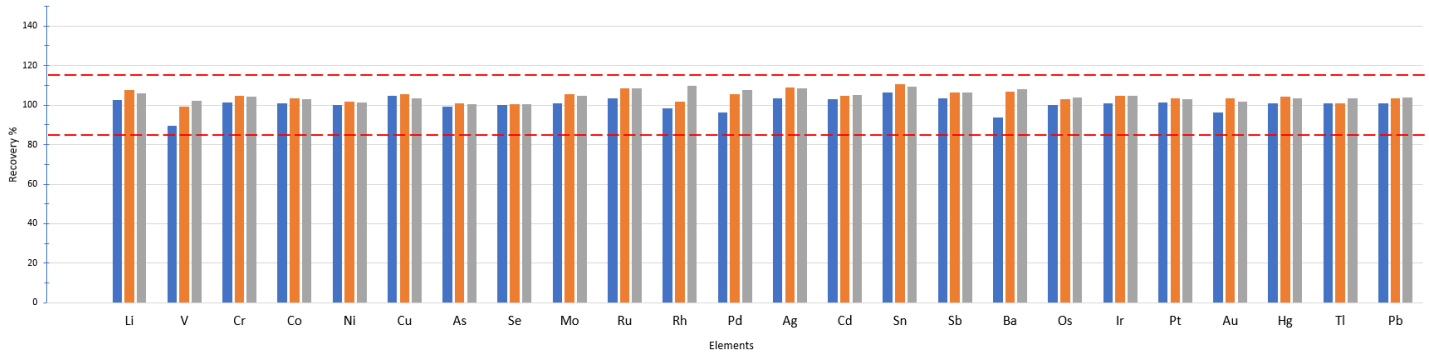

Figure 3. Accuracy results of spiked samples at 0.5 J (blue), 1 J (orange), and 1.5 J (grey).

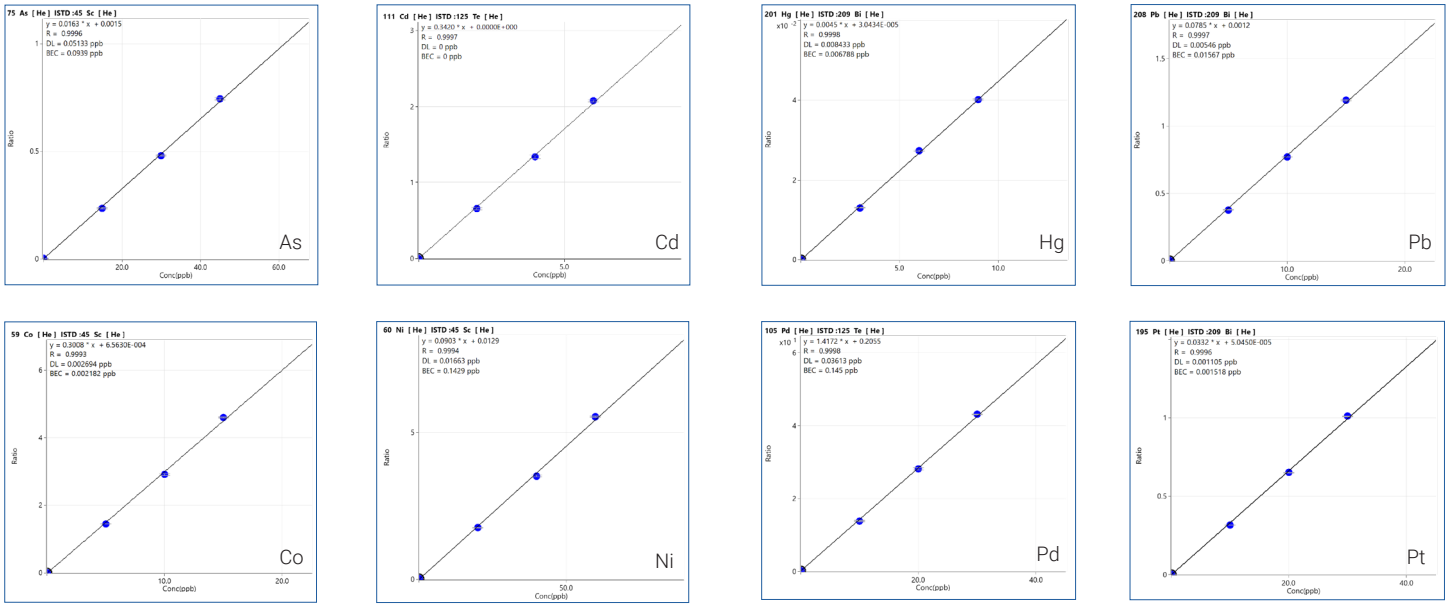

Figure 4. Calibration curves for As, Cd, Hg, Pb, Co, Ni, Pd, and Pt demonstrating low LOQs, good linearity, and wide quantification range.

Precision (repeatability)

Precision measurement procedures and acceptance criteria for internal precision are the same as those described in the Limit procedures precision section. The RSD values of less than 2.5% for all target elements in the 1 J spike samples confirm the excellent precision of the method (Table 4).

Intermediate precision (ruggedness)

Intermediate precision or ruggedness must be assessed by running the repeatability test again, either on a different day, on a different instrument, by a different analyst, or a combination thereof. The overall precision (n=12) must not exceed 25% RSD. The results of the intermediate precision test of 12 independent 1 J spiked samples run on a different day and by a different analyst are shown in Table 4. The intermediate precision was less than 4% RSD, which was well within the acceptance criteria. The excellent repeatability and intermediate precision results highlight the reliability and ease-of-operation of the 7850 ICP-MS for the routine analysis of oligonucleotides.

Conclusion

The study has shown the suitability of the Agilent 7850 ICP-MS for the identification and quantification of 24 regulated elements in a synthetic antisense oligonucleotide sample in compliance with existing USP and ICH guidelines for drugs. As the development and use of oligonucleotides in therapeutic applications continue to advance, ICH/USP and FDA regulatory guidelines that are specific to oligonucleotide products are expected to be published.

A preset USP/ICH method in the ICP-MS MassHunter software simplified method development by providing predefined operation parameters, a pre-optimized data processing method, and preloaded performance limits. The instrument was autotuned. Autotuning also saves method development and daily setup time, especially for new or inexperienced users. All elements were measured using a single data acquisition mode, with effective removal of polyatomic interferences ensured by operating the ORS[4] collision cell in He-KED mode to enhance the specificity of the measurements.

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). The results confirmed the system suitability of 7850 ICP-MS for the elemental impurities analysis of oligonucleotides, an emerging class of biotherapeutics.

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