Determination of Chromium in Gelatin Capsules using an Agilent 7700x ICP-MS

Application note

Pharmaceutical

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Introduction

Many medications that are administered orally are enclosed within capsules. Frequently both hard-shelled and soft-shelled capsules are made from edible animal protein (gelatin) which is prepared from various animal by-products such as bone and skin. The *Pharmacopoeia of the People's Republic of China* (2010 version) sets a clear standard for the grade of gelatin that can be used for drug capsule production and requires that pharmaceutical companies only purchase capsules from manufacturers that are licensed [1]. There have been recent reports that some companies in eastern China have been making and selling capsules made from cheaper industrial gelatin prepared from discarded leather [2]. Chromium, which is a known carcinogen, and can be toxic if ingested in large quantities, is used in the leather tanning process. Consequently, 20 to 90 times more Cr is typically found in the leather-derived gelatin than in pharmaceutical/edible grade gelatin. As a result, there is a need for a routine, highly sensitive method to

determine Cr in pharma sample types in order to restore confidence within the industry and with consumers. An ICP-MS method using collision/reaction cell technology is described below.

Experimental

Instrumentation

An Agilent 7700x ICP-MS with Octopole Reaction System (ORS^3) fitted with a low-flow concentric quartz nebulizer was used for all measurements. The objective was to use the ICP-MS to determine a full range of elements, in addition to Cr. The ORS^3 was operated in helium collision mode (He mode) only, which is effective at removing a wide range of plasma and matrix-based polyatomic species using kinetic energy discrimination (KED). Because He mode does not react with any analytes, and does not create any new interferences, elements that do not suffer from polyatomic interferences were also analyzed using He mode. Instrument operating conditions are summarized in Table 1.

Reagents and materials

HNO_3 (BASF); ultrapure water (18.2 MΩ) produced by Milli-Q ultrapure water systems; 100 g/mL of multi-element internal standard solution containing ^{6}Li, Sc, Ge, Rh, In, Tb, Lu and Bi (Agilent Part # 5188-6525); multi-element calibration standard STD-2A solution (Agilent Part # 8500-6940) containing Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, As, Cd and Pb.

Table 1. ICP-MS operating parameters

Parameter	Value	Parameter	Value
RF power	1300 W	Sampling depth	8 mm
Flow rate of plasma gas	15.0 L/min	Integration time	0.3 s
Flow rate of carrier gas	1.0 L/ min	No. of replicates	3
Flow rate of makeup gas	0.2 L/min	Sample uptake rate	0.4 mL/min
Spray chamber temperature	2.0 °C	Cell gas: helium	4.0 mL/min

Sample preparation

The gelatin capsules (Zhejiang IDC) were prepared by microwave digestion using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) as the digestion solution [3]. Approximately 0.3 g of each capsule was digested in duplicate with 4 mL of HNO_3 and 2 mL of H_2O_2 in pre-cleaned HF-100 microwave sample vessels. The filled vessels were placed on a 16-position rotating tray with a sensor positioned in one of the samples to monitor the pressure and temperature inside the sample container. In addition, an external IR sensor provided the temperatures for each individual sample in the tray. The digestion program consisted of 21 minutes of heating and 15 minutes of cooling, as shown in Table 2. All the samples were dissolved completely, resulting in clear solutions which were diluted to a final volume of 50 mL with 1% (HNO_3). Blanks, consisting of the acid mixture, were taken through the same microwave digestion program as the samples.

Table 2. Microwave digestion heating program for capsules

Step	Temp (°C)	Ramp (min)	Hold (min)
1	160	3	5
2	180	3	5
3	200	3	5
4 (cooling)			15

Results and discussion

Interference elimination

In this study, we investigated the use of collision cell technology using helium gas to eliminate interferences generated by the plasma and the sample matrix. Typical matrix-derived polyatomic interference ions arising from the analysis of gelatin capsules include $^{40}Ar^{12}C^+$, $^{51}V^1H$, $^{35}Cl^{16}O^1H$ and $^{40}Ca^{12}C$, etc. on ^{52}Cr and $^{40}Ar^{13}C$, $^{37}Cl^{16}O$ and doubly-charged $^{106}Ru^{++}$ on ^{53}Cr. The total dissolved solids (TDS) of the samples was less than 0.2% and any matrix effects were eliminated via the use of the internal standard calibration method.

Calibration curves

The Agilent 7700x ICP-MS was calibrated using an external standard method. A mixed standard calibration solution containing Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, As, Cd, Mo, Sn, Sb and Pb was prepared in

5% HNO_3 with a concentration range of 0, 10, 40, 100, 1000 µg/L (ppb). ^{103}Rh [~50 ng/mL] was added online as an internal standard. A typical calibration for ^{52}Cr is shown in Figure 1. The coefficient of linearity (r) is equal to 0.9999, instrument detection limit (IDL) is 0.011 µg/L and the background equivalent concentration (BEC) is 0.13 µg/L.

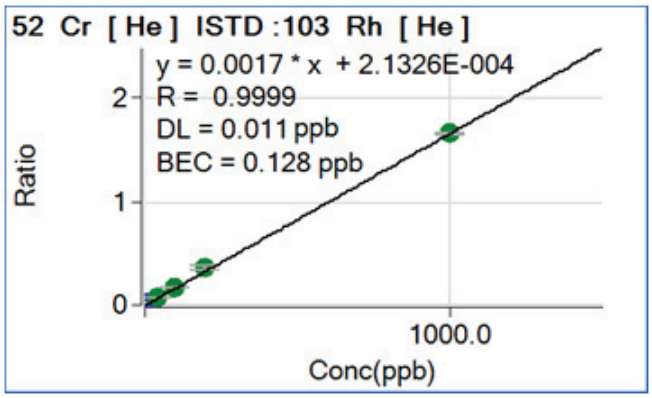

Figure 1. Calibration curve for ^{52}Cr

Instrument stability and recovery

To test the robustness and stability of the method, one sample (#1417) was analyzed repeatedly over 2 hours. The relative standard deviation (%RSD) was 2.1% and the recovery of a standard addition spike is presented in Figure 2. Twenty four samples were spiked with 5 µg/L and the recoveries ranged from 90% to 110%.

Method detection limits

Seven blank samples prepared using microwave digestion were analyzed to evaluate 3 sigma method detection limits (MDLs) for each element. The MDL for Cr was calculated as 0.12 µg/L (0.02 mg/kg in the original sample) which easily satisfies the requirements of *Chinese Pharmacopoeia* (2010 version) of < 2 mg/kg (ppm) [1].

Semi-quantitative results

Prior to carrying out quantitative analysis, the Agilent 7700x was used to scan the samples in semiquant mode. With the ORS^3 operating in He mode, the 7700x can produce high quality semi-quantitative (SQ) data from any unknown sample matrix, making it ideal for the screening analysis of large numbers of samples. The SQ scan was performed in 30 seconds and the data for a selected sample (S1) is given in Table 3.

The results show that the Ti content in sample "S1" was 34.61 ppm, which is equivalent to 8652 mg/kg (ppm) in the solid sample, suggesting a large quantity of TiO_2 was present in the capsule. If necessary, HF [1 mL] can be added to remove TiO_2, to give a clear solution, without affecting the Cr result.

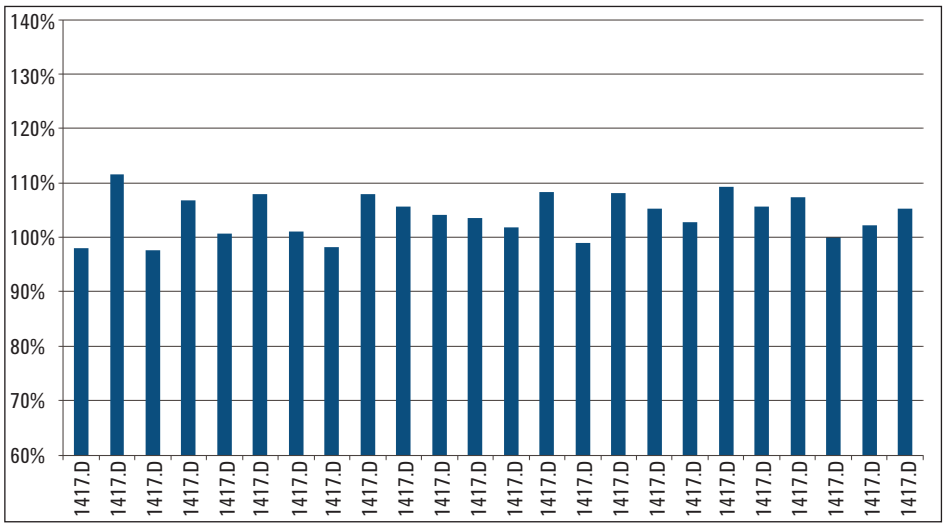

Figure 2. Recoveries of spikes added to 24 digests of sample "1417".

Table 3. Semi-quantitative concentration data (solution as analyzed) for the full suite of elements. Note, concentration units are automatically determined and vary according to measured sample concentration.

	Sample S1	Unit		Sample S1	Unit		Sample S1	Unit
7 Li	<212.209	ng/L	72 Ge	144.33	ng/L	146 Nd	388.03	ng/L
9 Be	<256.555	ng/L	75 As	1.05	µg/L	147 Sm	<5.153	ng/L
11 B	3.50	µg/L	78 Se	1.27	µg/L	153 Eu	7.52	ng/L
12 C	190.79	mg/L	79 Br	13.45	µg/L	157 Gd	35.19	ng/L
14 N	11535.71	mg/L	85 Rb	57.63	ng/L	159 Tb	<0.520	ng/L
23 Na	26.04	mg/L	88 Sr	24.61	µg/L	163 Dy	26.18	ng/L
24 Mg	865.93	µg/L	89 Y	49.90	ng/L	165 Ho	1.44	ng/L
27 Al	25.16	µg/L	90 Zr	20.23	µg/L	166 Er	17.92	ng/L
28 Si	6.87	µg/L	93 Nb	100.78	µg/L	169 Tm	2.46	ng/L
31 P	979.42	µg/L	95 Mo	988.16	µg/L	172 Yb	<1.957	ng/L
34 S	18.36	mg/L	101 Ru	<6.883	ng/L	175 Lu	<0.735	ng/L
35 Cl	2.04	mg/L	103 Rh	132.00	µg/L	178 Hf	541.63	ng/L
39 K	531.19	µg/L	105 Pd	<5.458	ng/L	181 Ta	3.03	µg/L
43 Ca	2.50	mg/L	107 Ag	105.83	ng/L	182 W	1.03	ng/L
45 Sc	8.02	µg/L	111 Cd	86.83	ng/L	185 Re	<1.604	ng/L
47 Ti	**34.61**	**mg/L**	115 In	14.79	ng/L	189 Os	<4.290	ng/L
51 V	655.70	ng/L	118 Sn	1.30	µg/L	193 Ir	14.62	ng/L
52 Cr	16.10	µg/L	121 Sb	46.79	µg/L	195 Pt	<2.552	ng/L
55 Mn	2.89	µg/L	125 Te	<265.435	ng/L	197 Au	12.03	ng/L
56 Fe	316.69	µg/L	127 I	1.65	ng/L	202 Hg	575.13	ng/L
59 Co	294.35	ng/L	133 Cs	146.18	ng/L	205 Tl	6.18	ng/L
60 Ni	3.24	µg/L	137 Ba	31.57	µg/L	208 Pb	5.03	µg/L
63 Cu	31.07	µg/L	139 La	208.86	ng/L	209 Bi	502.23	ng/L
66 Zn	22.40	µg/L	140 Ce	695.44	ng/L	232 Th	83.31	ng/L
69 Ga	8.49	µg/L	141 Pr	103.24	ng/L	238 U	536.74	ng/L

Quantitative results for chromium

Table 4 shows the quantitative results for chromium in 48 different sample digests. The concentration of ^{52}Cr in samples 1396, 1400, 1402, 1419, 1423, and 1436 exceeds 100 mg/kg which is much higher than the maximum value of 2 mg/kg (ppm) for Cr specified in the *Chinese Pharmacopoeia* (2010 version). In fact, only 20 samples met the 2 mg/kg requirement, which equates to a pass rate of 41.6%.

Table 4. Full quantitative analysis results for ^{52}Cr in 48 gelatin capsule sample digests

		^{52}Cr		^{103}Rh			^{52}Cr		^{103}Rh
Sample	Dilution factor	Concentration [mg/kg]	CPS RSD(%)	CPS RSD(%)	**Sample**	Dilution rate	Concentration [mg/kg]	CPS RSD(%)	CPS RSD(%)
1393	208.42	4.71	0.30	0.57	**1417**	196.2	0.78	0.81	0.08
1394	207.56	2.62	0.23	0.42	**1418**	223.21	90.04	0.77	0.61
1395	221.14	62.77	0.71	0.30	**1419**	195.2	168.9	2.03	1.95
1396	178.89	101.8	0.71	1.18	**1420**	213.49	1.18	1.36	1.24
1397	185.25	2.15	0.49	0.84	**1421**	201.29	1.66	0.10	0.44
1398	202.92	86.34	0.69	0.69	**1422**	235.96	80.36	1.36	1.73
1399	188.68	30.90	1.03	1.01	**1423**	222.22	110.5	0.45	1.30
1400	194.78	181.8	1.59	1.19	**1424**	196.46	75.65	0.64	0.13
1401	199.84	58.17	1.12	1.61	**1425**	215.80	1.82	0.53	2.27
1402	207.13	104.5	1.15	2.08	**1426**	211.15	1.86	0.43	0.61
1403	210.88	74.85	0.39	0.91	**1428**	208.07	1.38	0.58	0.44
1404	197.16	1.32	0.47	0.16	**1429**	227.07	1.55	0.73	0.77
1405	225.12	1.43	0.57	0.26	**1430**	203.67	1.37	0.42	1.38
1406	193.80	1.24	0.59	0.88	**1431**	216.83	69.32	0.27	0.26
1407	200.24	84.84	0.77	0.90	**1432**	197.86	7.17	0.30	0.82
1408	215.80	0.50	2.79	0.89	**1433**	216.36	11.90	0.62	0.28
1409	182.42	3.39	0.59	2.83	**1434**	210.61	5.11	0.77	0.99
1410	215.33	0.54	0.86	0.34	**1435**	225.43	0.76	1.04	0.25
1411	192.90	78.71	1.13	1.05	**1436**	218.44	145.5	1.46	0.75
1412	219.78	102.7	1.34	1.00	**1437**	211.15	1.57	0.21	0.62
1413	209.82	9.11	0.92	0.80	**1438**	214.78	0.19	1.37	0.50
1414	205.00	57.54	0.51	0.65	**1439**	200.00	0.14	1.79	0.38
1415	218.44	16.92	0.75	1.75	**1440**	204.42	0.45	0.70	0.76
1416	219.68	0.67	0.21	1.15	**1441**	210.53	1.37	0.94	0.29

Conclusions

Operating the Agilent 7700x with ORS^3 in helium mode effectively removes polyatomic interferences via kinetic energy discrimination allowing the rapid, accurate, semi-quantitative screening of complex sample types, such as gelatin capsules. For each sample, semi-quantitative and full quantitative analysis can be performed simultaneously without the need to adjust the instrument. Specifically for the analysis of the gelatin capsules detailed in this study, the 7700x was used to quantify Cr in 48 different gelatin capsule digests. The concentration range of Cr in the samples ranged from 0.14 to 196.2 mg/kg. Only 41.6% (20 samples) of the 48 samples met the 2 mg/kg requirement for Cr specified by the *Chinese Pharmacopoeia* (2010 version).

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Further Information

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Agilent publication: Proposed new USP general chapters <232> and <233> for elemental impurities: The application of ICP-MS for pharmaceutical analysis, 5990-9382EN, 2011

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