Characterisation of the First “Speciated" Chromium Enriched Organically Bound Yeast Reference Material: ERM-BD213a

Sarah Hill, John Entwisle, Panayot Petrov and Heidi Goenaga Infante

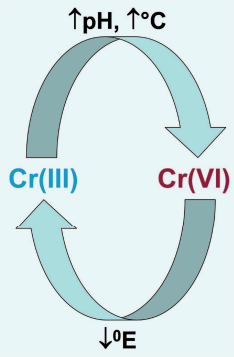
LGC Limited, Queens Road, Teddington, Middlesex, TW11 0LY, UK sarah.hill@lgcgroup.com

Background

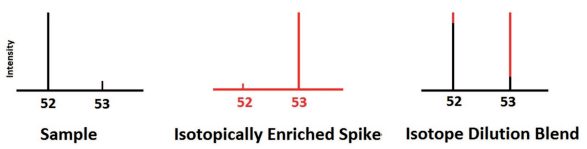
- Chromium (Cr) food supplements account for ~6% of mineral supplements sales
- Cr can act as essential element depending on its speciation
- **Trivalent chromium, Cr(III)**, is regarded as essential
- **Hexavalent chromium, Cr(VI)**, is classified as a Category 1 carcinogen[1]
- European Council maximum limit of <0.2% of the total Cr as Cr(VI)[2] in organically bound Cr enriched yeast for a total Cr concentration of 230-300 mg/kg
- Therefore, there is a clear need to accurately quantify total Cr and Cr species in complex food supplements

Introduction

- There is a lack of suitable certified reference materials available for Cr species in food and food supplements
- Therefore a new yeast-based reference material was produced: **ERM-BD213a**
- Both total Cr and Cr(III) are important target parameters
- **Isotope dilution analysis (IDA)** is the method of choice for reference value assignment:
  - SI traceability
  - high accuracy measurements
  - fit-for-purpose uncertainty
- Total Cr was determined by **double IDA** in combination with inductively coupled plasma mass spectrometry (ICP-MS)
- Cr species have complex chemistry and their determination is hampered by *bidirectional conversion* between Cr(III) and Cr(VI)
- Experiments with Cr(III) enriched yeast have shown ~68% of Cr(III) converts to Cr(VI) in basic media
- Single spike double IDA is not sufficient to account for these changes
- Therefore, a **species-specific double spike single IDA** method was developed
- It is based on isotope pattern deconvolution[3] which accounts for bidirectional species transformation in the analytical process
- Combined with chromatography and ICP-MS, Cr(III) can be accurately determined



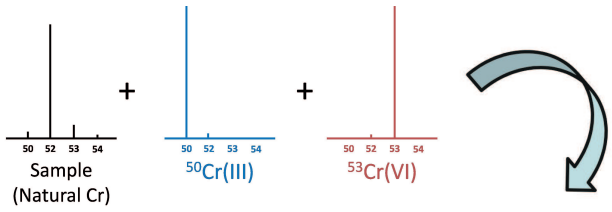
Methods - Double IDA



- A highly enriched isotope spike (^{53}Cr at 95.7% abundance) was added to the sample prior to sample preparation
- 0.2 g sample mixed with the ^{53}Cr enriched spike to give a gravimetric ratio (^{52}Cr/^{53}Cr) of 1
- 7 mL HNO_3 and 3 mL H_2O_2
- Microwave digestion @ 180ºC
- Diluted to 50 g with water
- The primary calibration standard (NIST SRM 3113a) was also prepared in the same manner
- Analysis using helium collision/KED mode (7700, Agilent Technologies)

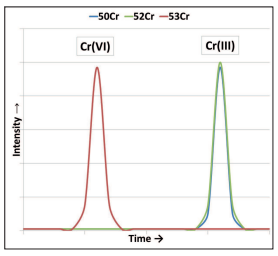
Methods - Species-Specific Double Spike Single IDA

- Two highly enriched Cr isotopes with different oxidation states were added to the sample prior to alkaline hydrolysis: ^{50}Cr(III) and ^{53}Cr(VI)
- This ensured species transformation was captured through the whole measurement process
- Highly selective separation of Cr(III) and Cr(VI) species within 20 mins using reversed phase ion pairing liquid chromatography (Bio-inert 1260, Agilent Technologies)
- Peak area determination of ^{50}Cr, ^{52}Cr & ^{53}Cr using ICP-MS/MS in ammonia reaction mode (8800, Agilent Technologies)
- *Isotope pattern deconvolution*[3] (IPD) was utilised to establish the degree of interconversion and quantification of the Cr species

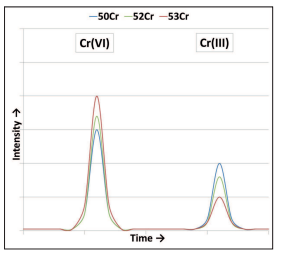


Alkaline Hydrolysis	0.1 g Sample, 1 M TPAOH & 94 mM EDTA, 5 h @ 95°C
Dilution	4 mM EDTA & 2.8 mM TPABr, pH 9.2, 1.5 h @ 85°C
Analytical column	PLRP-S 100 Å, 3 µm, 150 mm PEEK, ambient temp
Eluent	0.18 mM TPABr, 1mM EDTA, pH 9.2, 0.8 mL/min
Injection volume	50 µL

No species conversion



With species conversion

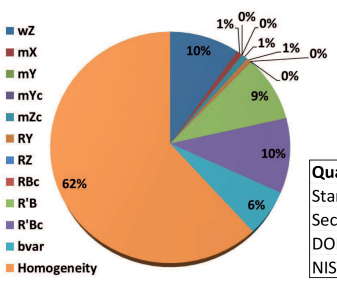


Results – Total Cr

$$w'_X = w_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_{Zc}}{m_{Yc}} \cdot \frac{R_Y - R'_B \cdot \frac{R_{Bc}}{R'_{Bc}}}{R'_B \cdot \frac{R_{Bc}}{R'_{Bc}} - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}} \cdot \frac{\sum R_{iX}}{\sum R_{iZ}}$$

- The characterisation was undertaken using 12 bottles in duplicate, with the analysis split over 2 days
- Quantification *via* double IDA using the equation above in accordance with ISO/IEC 17025 accreditation

Mass Fraction Total Cr	Expanded Uc	Relative Expanded Uc	Coverage Factor k (95% CI)
305.5 mg/kg	5.0 mg/kg	1.6%	2



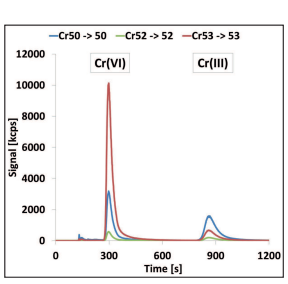
Quality Control	% Recovery
Standard Addition (n=2)	99.1
Secondary Standard (n=2)	100.0
DORM-4 (n=4)	101.5
NIST SRM 3280 (n=4)	106.2

Results – Cr Species

- The Cr species were determined using the species-specific double spike single IDA in combination with IPD calculation[3]
- 12 bottles in duplicate, fully nested design comprising of three runs
- Cr(VI) was not detected under strong reducing conditions induced by the matrix

Mass Fraction Cr(III)	Expanded Uc	Relative Expanded Uc	Coverage Factor k (95% CI)
302 mg/kg	47 mg/kg	15.6%	4.3

- The major uncertainty contribution was the batch-to-batch variation (94%) with the remainder attributed to homogeneity



Quality Control	% Recovery
Standard Addition (n=3)	95.3
Independent Standard (n=3)	99.2
Confirmatory Results	**mg/kg**
NIM China	292 ± 21

Conclusion

- **ERM-BD213a:** first reference material for total Cr and Cr species in supplements
- *Two different IDA* methods were used highlighting the power of the technique
- The total Cr value (305.5 mg/kg) was certified with methodology accredited to ISO/IEC 17025 with low uncertainty (1.6% relative)
- The IPD approach ensured (III)↔(VI) transformations were accounted for and the Cr(III) species accurately determined (302 mg/kg) with fit-for-purpose uncertainty (15.6% relative), demonstrating its capability for complex species
- This material represents an important step forwards to support challenging EU regulations[2]

References

[1] EU Publication SCOEL/REC/386 Chromium VI compounds (https://publications.europa.eu/s/iw4W)

[2] EC Directive 2002/46/EC (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02002L0046-20170726)

[3] J. Meija et al., J. Anal. At. Spec., 2006, 21, 1294–1297

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