

# DETERMINATION OF TOTAL SELENIUM AND SELENOMETHIONINE IN COW MILK BY HPLC COUPLED TO ICP-MS



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## Introduction

Selenium is an essential trace element for human health and has a recommended dietary allowance (RDA) for adults of 55 µg per day. Moreover, there is current evidence that intakes higher than the RDA (up to 200 µg per day) can protect the human body against free radicals, which can cause degenerative and age-related diseases as well as certain types of cancer. Recently, published data show that the benefits of Se depend on the form in which it is ingested. Estimation of adequate Se levels in the human diet requires information not only on total Se but also on the bioaccessible species in the sample. The aim of this work was to evaluate the concentration of total selenium and selenomethionine in cow milk.

## Material and Methods

Prior to the determination of total selenium, milk samples were subjected to a closed vessel microwave digestion to completely destroy the organic matter. In order to optimize this step of sample preparation two different methods (1 and 2), varying H<sub>2</sub>O<sub>2</sub> ratio, time and temperature conditions were tried to avoid the problem of spectral interferences of selenium a Dynamic Reaction Cell (DRC-ICP-MS) was used. Results were evaluated by using the determination of Isotopic Dilution (ID-ICP-MS) compared with the standard addition. Repeatability and trueness of the method for the determination of total selenium were evaluated using two reference materials, SRM-1549 and BCR-063R.



Table 1 – ICP-MS conditions total selenium

	Method 1	Method 2
Forward Power	1550 W	1400 W
Nebulizer flow	1.0 ± 0.2 L/min	0.82-0.85 ml/min
CCT	He : 4 ml/min	He/H <sub>2</sub> :
	H <sub>2</sub> : 4.9 ml/min	3.2 ml/min

Table 3 – Microwave digestion: Method 2

Step	Time (min)	Temperature (°C)	Power (W)
1	10	1000	180
2	5	0	180
3	6	1500	200
4	5	0	200
5	6	1000	90
HNO <sub>3</sub> : H <sub>2</sub> O <sub>2</sub>			5:1 7:1

Both enzymatic and acid hydrolysis of milk proteins were investigated as extraction procedures in view of Selenomethionine (SeMet) determination in milk.

Table 4 – RP-HPLC-ICP-MS conditions selenium speciation (Method 3)

Column	C18 RP-HPLC: 15 cm x 4.6 mm id x 4 µm
Mobile phase	0.1% PFPA in 2% CH <sub>3</sub> OH
Injection volume	100 µL
Flow rate	1.0 ml/min

Table 2 – Microwave digestion : Method 1

Step	Time (min)	Power (W)
1	10	220
2	5	0
3	10	440
4	5	0
5	6	570
6	5	0
7	6	220
HNO <sub>3</sub> : H <sub>2</sub> O <sub>2</sub>		5:1



## Results

Table 5 – Certified values of total selenium (µg/kg)

	Value	Uncertainty
BCR-063R (Indicative value)	129	-
SRM-1549	110	± 10

Figure 1 – Selenomethionine in several types of milk samples (retention time – 3 min) – enzymatic hydrolysis

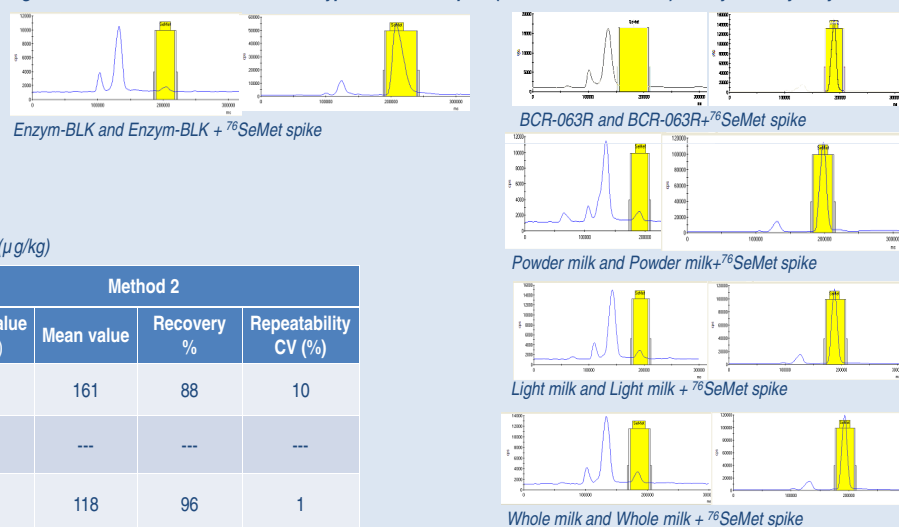


Table 6 – Concentration of total selenium (µg/kg)

	Method 1	Method 2			
	Mean value (CCT)	Mean value (CCT)	Mean value	Recovery %	Repeatability CV (%)
Milk Powder	141	164	161	88	10
BCR-063R	124	134	---	---	---
SRM-1549	---	139	118	96	1

## Conclusion

The presented work shows that both methods are suitable to determine selenium in milk since the results obtained for the two reference materials are in agreement with certified values. The conditions used in this work seemed effective to achieve accurate determination of total selenium and SeMet in this difficult matrix, but it is still necessary to optimize the conditions of Collision Cell Technology to obtain better results in the samples. Regarding the identification of selenomethionine, this study shows that this inorganic element is present in milk samples. For future work, methods for selenium's speciation will be developed in order to identify and quantify other important species.

## References

- [1] A. Alzate, M.C. Pérez-Conde, A.M. Gutiérrez, C. Cámara, Selenium-enriched fermented milk: A suitable dairy product to improve selenium intake in humans  
 [2] Martin Nash, Determination of Selenomethionine in Nutritional supplements using HPLC Coupled with XSeries// ICP-MS with CCT, Application Note: 40745, Thermo