BIOACCUMULATION OF TOXIC METALS IN FISH OILS CAPSULES

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*Toxic contaminants in fish oil are of particular interest because of the potential risks to humans. The permissible levels for heavy metals in drugs and dietary supplements established by the United States Pharmacopoeia are very low (traces, 0.1ppm). As opposed to the Pharmacopoeia tests for metals, a multielement ICP-MS method for Pb, Cd, As and Hg in fish oil supplements with various matrices has been developed. In order to confirm the data obtained the following parameters were taken into account for the validation of method: calibration (linear curves between 1-50 ppb), accuracy (above 96%), precision (*RSD $\leq$ 5 %*), detection limit (LOD) and quantification limit (LOQ). Additionally this work was carried out by scanning the entire mass range (TQ Spectrum) to identify all of toxic elements and bio elements. Heavy metal concentrations were bellow the limits of admissibility laid down in international regulations for the samples analyzed. The bioaccumulation of these toxins in fish oil capsule confirms the safety of long-term use of the supplements studied.*

Keywords: Toxic metals, fish oil, ICP-MS, Total Quant method

1. Introduction

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because most of them have toxic effects on organism. Among environmental pollutants, heavy metals are of particular concern, due to their potential toxic effect and ability to bio accumulate in aquatic ecosystems [1], [2].

Aquatic foods have essential amino acids, fatty acids, protein, carbohydrates, vitamins and minerals. Among sea foods, fish are commonly consumed and hence there is a connecting link for transfer of toxic heavy metals in human beings. Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish which, in turn, may enter into the human metabolism through consumption causing serious health hazards [3], [4].

Monitoring and control of impurities generally assures the quality and safety of a drug. The analytical studies on the evaluation of impurities in drugs

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are among the most important issues in modern pharmaceutical analysis [5]. In this context, United States Pharmacopeia [6] imposes severe limits for the analysis of inorganic impurities in various pharmaceuticals and medicines.

For fish oil capsules the limits of toxic contaminants expressed as maxim ppm content are as follows:

Pb - 0.1 ; Cd - 0.1 ; As -0.1 ; Hg - 0.1.

For other two products (fish oil enriched with vitamins and antioxidants), the legal permissible limits are different and have been set by the producer:

Pb - 3 ; Cd - 1 ; Hg - 0.1.

Many of heavy metals are toxic at only slightly elevated free ion concentrations [7], while others such as cadmium, lead and mercury have no known biological roles and are detrimental to essential life process [8].

Mercury occurs naturally as a mineral and is widely distributed throughout the environment as a result of natural and human activities [9]. Entering into water bodies the inorganic mercury is trapped in sediment particles where the sulfur–reducing anaerobic bacteria bio-transform the mercury into methyl mercury [10]. Due to bioaccumulation and bio-magnification properties through the aquatic food chain, longer lived and larger fish than smaller fish accumulates the highest levels of methyl mercury and consumption of this contaminated fish can lead to severe poisoning in both humans and wildlife [11], [12].

Arsenic has been considered as essential trace element for the normal growth and development of animals [13]. However, naturally occurring arsenic is found as a contaminant in drinking water. The accumulation and bio-magnification of arsenic in marine flora and fauna are phenomena that have generated a great deal of interest in the nutrition and trade industries in recent years. The notorious association of arsenic with poisoning has led to many studies on the possible risks associated with human exposure.

Lead is a neurotoxin that causes behavioral deficits in vertebrates [14] and can cause decreases in survival, growth rates, learning and metabolism [15]. Young stages of fish are more susceptible to adults or eggs.

In animals, **cadmium** concentrates in the internal organs like kidney and liver rather than in muscle or fat. Cadmium levels usually increase with age. The acute toxicity of cadmium to aquatic organisms is variable. Cadmium interacts with the calcium metabolism.

Contaminants in fish can pose a health risk to the fish themselves, to their predators, and to humans who consume them [16], [17], [18].

There are a few data in the literature on the determination of heavy metals in fish oil. Little number of data provided from United States Pharmacopeia mono-elements refers to their determination by graphite furnace atomic

absorption spectroscopy (GFAAS) or refers to the determination of heavy metals in fish species [19], [20].

The objectives of this study were to quantify the concentration (at trace level) of total Pb, Cd, As and Hg by means of ICP-MS multi-element method, in pure fish oil or associated with vitamins and antioxidants, after microwave digestion. Unlike pharmacopeia tests referring to graphite furnace absorption spectrometry, this technique can be easily automated and hence it is a perfect tool for fast routine analysis [21].

2. Experimental

2.1. Reagent and sample preparation

Ultrapure water with a specific resistance of 18 MΩ or greater was obtained using a Milli-Q^{TM} water purification system (Millipore Corporation, USA).

Nitric acid (65%, m/v, Ultrapure® grade, Merck, Darmstadt, Germany) was used. Standard solutions 10 mg L^{-1} of As, Cd, Pb and Hg (traceable to NIST) were procured from Perkin Elmer, Inc. Shelton, USA.

Standard solutions for calibration curves were prepared by dilutions of 10 mg L^{-1} of As,Cd, Pb and Hg procured from Perkin Elmer, Inc. Shelton, USA. Solutions prepared had a content of lead, cadmium, arsenic or mercury in the range of 1-40 ppb, corresponding to the concentration range 0.001 to 0.040mg L^{-1}.

The samples were completely dissolved by digestion, resulting in clear solutions. The contents were cooled to <50 °C before opening the vessels and quantitatively transferred to 25 mL tubes. Then the contents were cooled to room temperature and diluted to 20 mL with deionized H_2O prior to analysis.

2.2. Instrumentation

Microwave Digestion System. Microwave digestion of the fish oils samples for ICP-MS analysis was carried out using a model Multiwave™ 3000 microwave system (Anton Paar) equipped with a Pressure/Temperature (P/T) Sensor Accessory. The system has a power feedback control that applies ramped microwave energy to achieve a user-selected power program and pressure monitoring to prevent pressure build-up.

ICP-MS model PerkinElmer® ELAN DRC-e ICP-MS quadrupole-based ICP-MS was used for the determination of As, Cd, Pb, and Hg. Detailed analytical conditions for measuring the isotopes are given in Table 1.

Table 1

ELAN DRC-e ICP-MS Instrumental Parameters

Parameter	Value
RF power	1100 W
Plasma gas flow	15 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.96 L/min
Nebulizer	MEINHARD® Concentric Type A3
Spray chamber	Baffled Quartz Cyclonic
Scanning mode	Peak Hopping
Dwell time	50 ms
Replicates	5
Integration time	1000 ms
Detector	Dual
Isotopes	^{75}As, ^{114}Cd, ^{208}Pb, ^{202}Hg

2.3. Validation of the ICP-MS developed method

The ICP-MS method was validated according to the European Pharmacopeia 8 (2012) criteria [22], [23].

Linearity and range

The linearity was evaluated by running of six calibration standard solutions corresponding to the concentration range 0.001 to 0.040 mg / L of Pb, Cd, As, Hg.

The calibration curves were processed by using the soft of ICP, calculating the mean and relative standard deviation for the lowest and for the highest calibration level and respectively, the correlation coefficients.

Repeatability

The precision was assessed by performing six determinations (5 replicates each) on a homogeneous sample. Concentrations were calculated based on mean, standard deviation (s) and the relative standard deviation (RSD) for lead, cadmium, arsenic and mercury.

Accuracy

The accuracy was calculated as the ratio recovery of a known quantity of impurities added to the sample of the fish oil. Recovery was evaluated analyzing 3 samples of fish oil - soft gelatin capsules spiked with the following quantities: 0.08 ppm lead, 0.03 ppm 0.03 ppm cadmium and arsenic.

Limit of detection (LOD) and limit of quantification (LOQ)

Method Detection Limits were obtained from the standard deviation of 7 measurements of the reagent (LOD) blank for each element.

It was verified that the limit of quantification (LOQ) for Pb, As, Cd and Hg (for example, determined using 10σ approach) is bellow the value to be measured.

2.4. Total Quant Spectrum

A screening was performed by the Total Quant method. A full range of masses were scanned to identify all the elements present in fish oil pure, respectively associated samples. Additionally a detailed mass spectrum was performed within the interested range (9-100 amu) for each type of matrix.

3. Results and discussion

ICP-MS offers many analytical advantages, including low detection limits with wide dynamic range, high sample throughput and multi-element capability. For the determination of heavy metals, the samples were collected from market.

Table 2 shows the results of three fish oil caps with the legal permissible limits set by USP. The variation in the content of toxic heavy metals demonstrates that there are a large number of factors involved in the quality of these supplements, including the sources of raw fish, contamination from water and manufacturing procedures.

Table 2

The levels of inorganic impurities in three sample of fish oil caps with the legal permissible limits set by USP 2012. (Units: ppm)

Element	m/z	P1	LPL	P2	LPL	P3	LPL
As	75	0.0030	0.1	-	-	-	-
Cd	114	0.0058	0.1	0.0038	1.0	0.0031	1.0
Hg	202	0.0049	0.1	0.0019	0.1	0.0015	0.1
Pb	208	0.0960	0.1	0.1128	3.0	0.1170	3.0

P1 – Fish Oil
P2 – Fish oil + E vitamin
P3 - Fish oil + E vitamin and antioxidants
LPL – Legal Permissible Limit

Validation study

Linearity was verified according to the protocol provided by European Pharmacopeia 7 validation. Solutions were prepared containing lead, cadmium, arsenic, or mercury in the range of 1-40 ppb, which corresponds to the concentration range from 0.001 to 0.040 mg / L. The results obtained are collected in the table 3.

Table 3

Linearity for lead, cadmium, arsenic and mercury

Nr. crt.	Pb		As		Cd		Hg	
	Concen tration	RSD %	Concen tration	RSD %	Concen tration	RSD %	Concen tration	RSD %
1.	1.0	0.7	1.00	1.2	1.00	1.1	1.0	0.9
2.	1.4	0.7	1.93	1.7	1.98	0.5	1.6	1.3
3.	4.7	1.0	4.93	4.7	4.95	5.0	5.1	0.7
4.	9.3	0.8	10.00	1.2	10.07	0.8	12.2	1.7
5.	19.5	1.1	20.09	1.8	20.20	1.2	22.3	0.7
6.	39.6	0.7	39.96	0.6	39.99	0.6	-	-

The following results are obtained:

• the correlation coefficient R is 0.99948 for lead, 0.99995 for cadmium, 0.99998 for arsenic and, respectively, 0.99808 for mercury. Therefore, the correlation coefficient R meets the requirements for admissibility: R> 0.99;

• the residual value for each standard calibration is distributed randomly around the calibration curve;

• the ratio between the relative standard deviation for the lowest and the highest standard is between 0.5-2.0, the values being 1.0 for lead, 1.8 for cadmium, 2.0 for arsenic, 1.3 mercury, therefore the method is linear in 0.001-0.04 ppm range.

Repeatability

In a homogeneous mixture of oil were made over six measurements each of 5 readings (P1, P2, P3, P4, P5, P6). The results on concentrations, mean, standard deviation(s) and the relative standard deviation (RSD) for lead, cadmium, arsenic and mercury are collected in the table 4.

Table 4

Repeatability for lead, cadmium, arsenic and mercury

Nr.crt.	Element concentration (ppm)			
	Lead	Cadmium	Arsenic	Mercury
1	0,0972	0,0137	0,0098	0,0093
2	0,0903	0,0153	0,0107	0,0104
3	0,0920	0,0135	0,0103	0,0106
4	0,0977	0,0140	0,0105	0,0099
5	0,0944	0,0139	0,0109	0,0095
6	0,0955	0,0134	0,0102	0,0100
Mean	0,09452	0,01400	0,01040	0,00995
s	0,00291	0,00070	0,00039	0,00050
RSD	3,08	4,95	3,75	4,98

One may conclude that the results obtained for RSD are 3.08% for lead, 4.95% for cadmium, 3.75% to 4.98% for arsenic and mercury, RSD values obtained are included in the admissibility condition (RSD ≤ 5 %), therefore the method is precise.

Accuracy

To determine this parameter retrieval yields were calculated from 3 samples of fish oil - enriched with the following quantities: 0.08 ppm lead, 0.03 ppm cadmium, 0.003 arsenic and 0.03 ppm mercury. The recovery data obtained are shown in the Table 5 being on acceptable levels (from 96 to 105%)

Table 5

Recovery for lead, cadmium, arsenic and mercury

lement	Measured concentration	Concentration after fortification	Recovery %
s	0.0103±0.0005	0.0404±0.0026	99.006±5.665
d	0.0142±0.0010	0.0502±0.0024	104.176±3.082
b	0.0932±0.0036	0.1639±0.0031	96.21±3.492
g	0.0101±0.0007	0.0388±0.0009	103.237±1.525

One may conclude that mean recovery yields are 96.21% for lead, 104.17% for cadmium, 99.01% for arsenic and 103.237% for mercury. The yield values being within the range of admissibility 80-120%, the method is accurate.

Method Detection Limits (MDL) were obtained from the standard deviation of 7 measurements of the reagent blank, and the MDL for each element was found to be 0.0001 μg / L Pb, 0.0001 μg / L As , 0.0001 μg / L Cd and 0.0001 μg / L for Hg. The established MDL for these four elements are low enough to analyze and evaluate samples in order to meet the requirements of certain established guidelines.

Total Quant method

Fish oil samples, pure or in combination with dietary supplements, have been analyzed by the Total Quant method [24].

Detailed mass spectra for interested range (9-100 amu) are presented in Fig. 1 for pure fish oil, respectively for the associated oil in Fig. 2.

By scanning the entire mass range (Total Quant method) it was shown that in pure fish oil P1 there are metals with bio element role (at trace level). Their

concentration increases with the addition of vitamins and natural antioxidants, in P3, especially for Na, Mg, K, Fe, Ca.

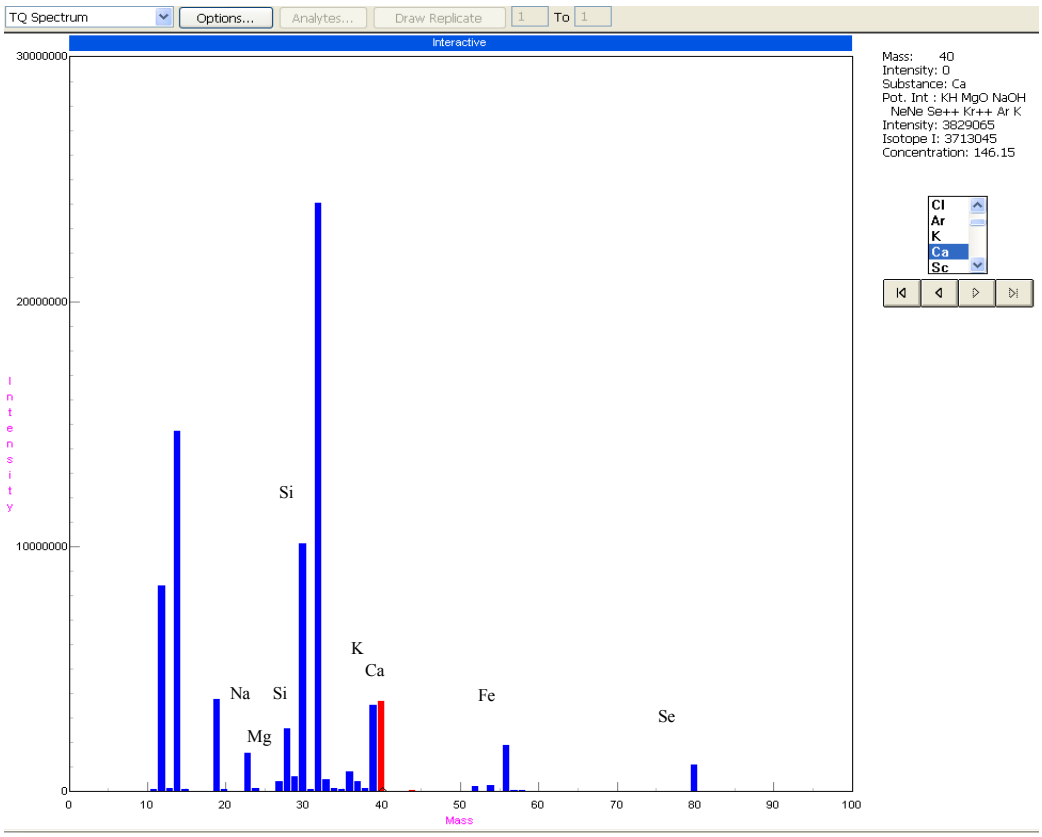

Fig. 1. Mass Spectra Total Quant (9-100 amu) – detailed for fish oil (pure)

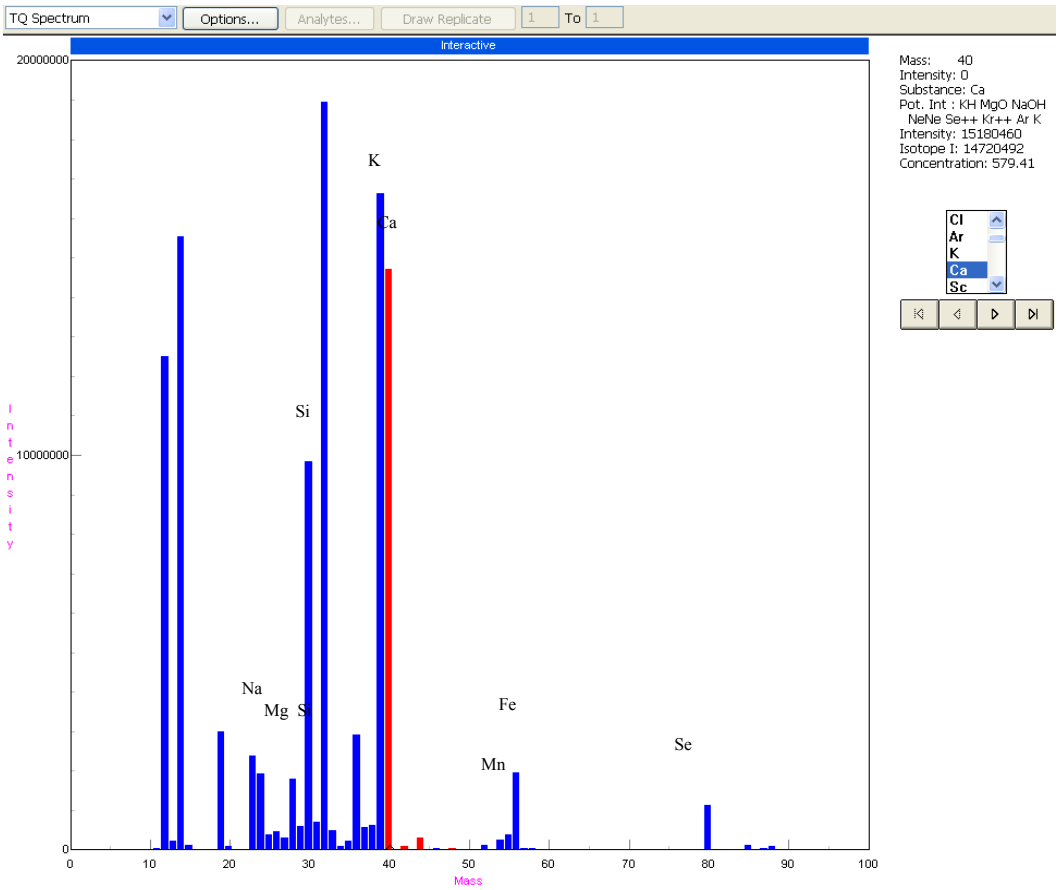

Fig. 2. Mass Spectra Total Quant(9-100 amu) - detailed for fish oil associated

4. Conclusions

A systematic study on monitoring the toxic impurities in fish oil capsules has been performed.

A suitable ICP-MS method was developed and validated for simultaneous quantitation of four toxic contaminants in fish oil supplements with different matrices.

The method is an excellent tool for multi-element trace evaluation since combines high sensitivity and superior detection limits with easy of use and high sample throughput.

The method was successfully applied for trace detection of four toxic elements: Pb, As, Cd, and Hg.

Heavy metal concentrations were bellow the limits of admissibility laid down in international regulations for the samples analyzed. It was also

demonstrated that the bioaccumulation of these toxins in fish oil capsules confirms the safety of long term use of these supplements studied.

The developed methodology (ICP-MS and TQ) can be successfully used as a valuable instrument in order to identify both toxic metals and beneficial bio elements existent in different drugs and dietary supplements according to European requirements.

R E F E R E N C E S

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