Laboratory of LC-MS/MS for the Pharmaceutical Engineering, second degree

Determination of inorganic chemical forms of selenium in dietary supplements

The aim of the laboratory is to get acquainted with the possibilities of using modern tandem mass spectrometry and combined LC-MS/MS technique, to acquire the basics of analysis and interpretation of mass spectra, as well as the use of the technique for qualitative-quantitative analyses.

Notes:

1. Any operation of the apparatus may only be performed by the class instructor, due to the highly complex nature of the equipment and control software.
2. The mass spectrometer operates at high voltages (e.g. acceleration voltage, voltage applied to the capillary) and therefore direct contact with exposed electronic components, wires, etc. should be avoided.
3. Care should be taken with the hot surfaces of the ESI ion source chamber while performing the exercise.
4. Be sure to wear protective gloves and goggles and an apron.
5. general chemical laboratory safety rules apply during the exercise

First part of the exercise - performing mass spectra and their interpretation

Topics to be prepared:

Principle of operation of electrospray ion source and tandem mass spectrometer

Apparatus: Mass spectrometer API 4000 QTRAP (Biosystems, MDS Sciex, USA), syringe pump, glass syringe, automatic pipettes, 1.5 mL Eppendorf tubes

Reagents: Sodium selenite (VI) and selenite (IV) standard solution, methanol, distilled water, ammonia water

Performing the exercise:

1. Familiarize yourself with the structure of the mass spectrometer and the design of the electrospray ion source.

2. Discuss and demonstrate the technique of introducing samples directly into the mass spectrometer - syringe.

3. Performance of spectra:

- selenite (VI) and selenite (IV) in aqueous-methanol solution and ammonia methanol solution,
- Perform a fragmentation spectrum for the selenium compound (one of two).

4. Interpret the obtained mass spectra with observation of the isotopic distribution of the selenium-containing compound.

5. Discuss the data obtained from the fragmentation spectrum.

Preparing the report:

1. In the description of the exercise, include details of the exercise, the results obtained, and any printouts received via email from the exercise instructor.

2. Interpret mass spectra and fragmentation spectra of selenium compounds (indicate the pseudomolecular ion, the isotope distribution of this ion, propose structures of the resulting fragments in the fragmentation spectrum).

The second part of the exercise - identification and determination of selenium in a dietary supplement.

Issue to prepare:

Principle of operation of a tandem mass spectrometer with ESI type ionization, general information on liquid chromatography (mobile phase, stationary phase, apparatus construction, isocratic elution, gradient elution, basic parameters used in qualitative and quantitative analysis).

Apparatus: Liquid Chromatograph coupled to an API 4000 QTRAP tandem mass spectrometer

Reagents:

- 25-50 mL beakers,
- 1 mL volumetric flasks,
- pipettes,

- 1.5 mL vials,
- methanol,
- ammonia water,
- standard solutions of selenate(VI) and selenate(IV),
- medical syringes with needles,
- syringe filters,
- mortar,
- ultrasonic cleaner,
- laboratory centrifuge,
- analytical balance,
- dietary supplement.

Performing the exercise:

1. Preparation of standard solutions:

From a standard solution containing 5 µg/mL each of selenate(VI) and selenate(IV), prepare a series of standard solutions at concentrations of: 0.05 µg/mL; 0.1 µg/mL; 0.25 µg/mL; 0.5 µg/mL; and 1 µg/mL.

The dilutions should take into account the composition of the mobile phase used for chromatographic separation (99% 5 mM NH_3 aq and 1% methanol).

2. Preparation of the supplement for analysis:

A. Weigh the tablet on an analytical balance.
B. Grind the tablet in a mortar.
C. Weigh the grated tablet.
D. Pour 10 mL of distilled water over the weighed sample of the grated tablet.
E. Place the sample in an ultrasonic cleaner for a period of 15 minutes.
F. Transfer the suspension to a centrifuge tube.
G. Centrifuge the sample for 15 minutes.
H. Filter the solution over the precipitate through a syringe filter with a pore diameter of 0.45 µm, and then again through a syringe filter with a pore diameter of 0.22 µm.

I. Based on the selenium content of the supplement, calculate the volume of sample to be taken for chromatographic analysis.

Note: in your calculations, assume that the theoretical content of selenium compound in the sample to be analyzed should be 0.25 µg/mL.

Make up the calculated volume of extract to 1 mL with mobile phase.

3. Perform the determination by LC-MS/MS technique:

The exercise leader will place the samples in an autosampler and start the process of their determination by the method whose conditions are described below:

Conditions for chromatographic separation

Chromatographic separation of the mixture of selenium compounds should be performed on a Luna C18 chromatographic column, as a mobile phase use a mixture of 5 mM aqueous solution of ammonia water and methanol. The flow rate of the mobile phase through the column was set to 0.20 mL/min. Conduct the separation at 35°C.

The operating parameters of the liquid chromatograph were collected in table 1.

Table 1: Chromatographic separation conditions

Measuring system component	**Operating parameters**		
Liquid chromatograph	UltiMate 3000 RSLC, Dionex		
Chromatographic column	Luna C18 C18 RP (150 mm x 2,1 mm i.d.), Thermo Scientifix		
Mobile phase	A: H_2O + 5 mM $NH_{3\ aq}$ B: MeOH		
Flow rate of mobile phase	O,20 mL/min		
Gradient	Time [min.]	% A	% B
	0	99	1
	1,5	99	1
	2,5	0	100
	3	0	100
Duration of analysis	3 min		

MS/MS detection conditions

Perform MS/MS detection of analytes using negative mode ionization. Ionize the sample by electrospraying. Detailed operating conditions of the mass spectrometer are shown in Table 2.

Table 2: Operating condition of the mass detector

Element	Operatin parameter
Mode of ionization	ESI
Mode of operation	Negative
Temperature [^{o}C]	550
curtain gas [psi]	30
nebulizer gas [psi]	40
auxiliary gas [psi]	40
Ion spray voltage [V]	-4500

Based on the fragmentation spectra performed for the selenite (VI) and (IV) standards, characteristic MRM pairs were selected and used to perform quantitative analyses. Then, the mass spectrometer operating parameters such as fragmentation potential and collision energy, which affect the sensitivity and selectivity of quantitative and qualitative analysis, were optimized for these pairs. Table 3 shows the mass spectrometer operating parameters used for qualitative and quantitative determination of selenium compounds.

Table 3: Optimal MS/MS operating parameters for selenium compounds

Compound	pseudomolecular Ion $[M-H]^{-1}$	Declustering potential [V]	MRM1	Collision energy [V]
selenium (VI) ^{80}Se	145	-50	145 → 128	-28
selenium (VI) ^{78}Se	143	-50	143 → 126	-28
selenium (VI) ^{82}Se	147	-50	147 → 130	-28
selenium (IV) ^{80}Se	129	-45	129 → 112	-32
Selenium (IV) ^{78}Se	127	-45	127 → 110	-32
selenium (IV) ^{82}Se	131	-45	131 → 114	-32

4. Based on the results obtained for the standard solutions and the sample of the supplement, identify the selenium compound contained in the supplement.

5. Determine the selenium content of the test supplement (use the standard curve technique or the multiple standard addition technique - to be decided by the instructor).

Report preparation:

1. Report the determined chemical form of selenium in the supplement.

2. Determine the content of the selenium compound in the supplement tablet.

3. Convert the selenium compound to elemental (selenium) content.

4. Compare the resulting selenium content and chemical form with that reported by the manufacturer.