

Identification and Quantification of Selenium Compounds in Sodium Selenite Supplemented Feeds by HPLC-ICP-MS

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Identifizierung und Quantifizierung von Selenverbindungen in Futtermitteln mit Natriumselenitzusatz durch HPLC-ICP-MS

1. Introduction

Selenium as a toxic and essential element has frequently been discussed in the past decades. It belongs to a group of micronutrient elements required in very small amounts by animals and humans for the basic functions of life (FORCHHAMMER and BOECK, 1991).

Before 1957 selenium was thought to be only highly toxic and carcinogenic. In 1957 SCHWARZ and FOLTZ observed that selenium-deficient food caused early death of rats (SCHWARZ and FOLTZ, 1957). A lot of medical complaints associated with a low Se intake were recognized, like protein energy malnutrition, haemolytic anemia, hypertension, ischemic heart disease, alcoholic cirrhosis, cystic fibrosis,

Zusammenfassung

Zur Identifizierung und Quantifizierung von Selenverbindungen in Futtermitteln wurde ein Anionenaustausch-Chromatographiesystem mit einem HP 4500 Induktiv-Gekoppelten-Plasma-Massenspektrometer gekoppelt. Die Trennung von Selenit, Selenat, Selenocystin und Selenomethionin wurde durch Hochdruckflüssigkeitschromatographie (HPLC) auf einer Hamilton PRP-X-100 Säule in 10 mM Citratpuffer (pH 5) erzielt, mit 2 % Methanol als mobiler Phase und einer Flußrate von 1.5 ml min^{-1} . Vier an der Grundlinie getrennte chromatographische Gipfel wurden innerhalb von 6 Minuten erhalten.

Verwendet man wäßrige Extrakte der Futtermittel, so lassen sich Selenit und manchmal auch ein schwaches Signal von Selenat identifizieren. Jedoch wurden nur 15–50 % der Gesamtkonzentration Selen gefunden. Dies läßt darauf schließen, daß ein Teil des Selenits zu weniger löslichen Formen in den Futtermitteln reagiert.

Verschiedene Extraktionstechniken und Extraktionsmittel, inklusive der enzymatischen Hydrolyse, wurden getestet, um den extrahierbaren Anteil von Selen möglicherweise zu erhöhen.

Schlagworte: Hochdruckflüssigkeitschromatographie, Anionenaustausch-chromatographie, Induktiv gekoppelte Plasma Massenspektrometrie, Selen, Futtermittel.

Summary

An anion exchange chromatographic system was coupled to an HP 4500 inductively coupled plasma mass spectrometer (ICP-MS) for the identification and quantification of selenium compounds in animal feeds.

Separation of selenite, selenate, selenocystine, and selenomethionine was achieved by high performance liquid chromatography (HPLC) on a Hamilton PRP-X100 column using a 10 mM citrate buffer (pH 5), 2 % methanol as mobile phase and a flow rate of 1.5 ml min^{-1} . Four baseline separated chromatographic peaks were obtained within 6 minutes. Using aqueous extracts of the feeds, selenite and sometimes a poor signal of respective selenate were identified. However, only 15 to 50 % of the total concentration of selenium was found. This suggests that part of the selenite was converted to less soluble forms in the feeds. Different extraction techniques and extraction solvents, including enzymatic hydrolysis were tested with respect to a possible enhancement of the extractable amount of selenium.

Keywords: High Performance Liquid Chromatography, Anion Exchange Chromatography, Inductively Coupled Plasma Mass Spectrometry, Selenium, Feeds.

infertility, cancer, arthritis, muscular dystrophy, and multiple sclerosis (HAYGARTH, 1994). In 1973 selenium was shown to be an integral part of the enzyme glutathione peroxidase (GSH-Px). This enzyme protects membranes from damage caused by the peroxidation of lipids (ROTRUCK et al., 1973). Peroxides are catalytically reduced to alcohols or water. Selenium has been claimed to protect from heart diseases and cancer because of its antioxidative properties (JACKSON, 1986). It prevents harmful effects caused by an excess of toxic elements such as arsenic or mercury.

Required amounts of selenium necessary for animal nutrition range from 0.04 to 0.1 mg kg⁻¹ feed depending on the animal species and the level of vitamin E in the diet amongst other factors (GIRLING, 1984). Vitamin E and selenium has been shown to interact in the prevention of liver and muscle degeneration in rats; large amounts of vitamin E decrease the need for as much dietary selenium and vice versa and selenium deficiency in animals depresses the immune system.

The selenium concentration in agricultural products is very low in many areas throughout the world. It has been traditionally attributed to poor supply of selenium from the soil, and ultimately the underlying geology. Selenium deficiency problems are obvious in large areas in Middle and Northern Europe (PFANNHAUSER, 1992a).

The concentration of selenium in Austrian soil has been determined in the "Austrian soil monitoring program". The average concentrations range from 0.22–0.35 mg kg⁻¹ Se (OBERÖSTERREICHISCHE BODENZUSTANDSINVENTUR, 1993; DANNEBERG, 1989; BURGENLÄNDISCHE BODENZUSTANDSINVENTUR, 1996). The average daily selenium intake of Austrians was found to be 35.5 µg Se per day, which is lower than the recommended "adequate and safe" intake of 50–200 µg Se per day (PFANNHAUSER, 1992b). 20–100 µg Se per day have been recommended as adequate by the German Society for Nutrition (RADKE, 1992).

The poor supply of selenium from soil has led to widespread selenium supplementation in the diets of livestock. Animals are supplemented with Se salts in feeds or salt/mineral mixtures. Because of its toxicity the selenium concentration in animal feeds may not exceed a maximum level. And on account of the essentiality the concentration should not fall below a minimum value. In feeds for ruminants the lower limit of selenium, where no deficiency diseases occur, lies at 0.1 mg kg⁻¹. Selenium concentrations higher than 5 mg kg⁻¹ may be toxic (SCHNITGER and LIECK, 1981). In the United States, supplementation of Se to animals is regulated by the Food and Drug Administration (FDA). The

allowable selenium supplementation level is to 0.3 mg Se kg⁻¹ for all major food-producing species. In the European Union the maximum allowable total concentration of selenium in animal feeds (natural and supplemented Se) is 0.5 mg Se kg⁻¹ (Federal gazette 1994: 273. ordinance: Feeds 1994).

Up to now animal feeds were only analysed with respect to their total concentration of selenium after complete mineralization of the matrix. The concentration of total selenium might be an important parameter with regard to deficiency or toxicity criteria. However, it cannot be an indicator for the biological availability of the element for animals and humans, because the chemical form and not the total concentration determines the beneficial effects, and toxic actions of a trace element. Therefore, the selenium compounds should be identified and quantified separately for more reliable indication of bioavailability. Interactions of various compounds mixed together with sodium selenite in complex feedstuffs have rarely been investigated.

High-performance liquid chromatography (HPLC) is an appropriate method for the separation of selenium compounds in extractant solutions. Since WHEELER and LOTT in 1974 first suggested the use of HPLC for the identification and quantification of selenium compounds (WHEELER and LOTT, 1974), numerous reviews have been published (KOELBL et al., 1993a; PYRZYNSKA, 1996; DAUCHY et al., 1994; OLIVAS et al., 1994). Usually either ion-exchange or reversed phase ion pairing chromatography were applied for the separation of ionic selenium compounds. Biological samples and feeds have been known to cause problems during the analysis, because they often contain, in addition to the selenium compounds of interest, many other extractable compounds in appreciable concentrations causing interferences. Selenium-specific detectors can considerably simplify the chromatographic process, because only the compounds containing selenium need to be separated. Additionally, even large excesses of selenium-free co-eluting substances usually do not interfere with such determination. Past examples of selenium-specific detectors coupled to chromatographic systems include GF-AAS (KOELBL et al., 1993b; POTIN-GAUTIER et al., 1993), and ICP-AES (LABORDA et al., 1991; HAGEGE et al., 1995). A substantial improvement in chromatographic detection of selenium species has been achieved by ICP-MS, which allows on-line detection of the separated selenium species at biological sample concentrations (GE et al., 1996; PEDERSEN and LARSEN, 1997; YANG and JIANG, 1995; GOESSLER et al., 1997).

In order to quantify the amounts of selenium readily available in feedstuffs, respective extracts at various physiological pH and enzymatic hydrolysis will be performed. Selenium will be specified in the extracts by coupling a HPLC with an ICP-MS as an element-sensitive detector.

2. Material and Methods

2.1 Instrumentation

A HP 4500 inductively coupled plasma quadrupole mass spectrometer (Hewlett-Packard, Germany) was used as selenium-specific detector. The hydrogen selenide was produced in a CETAC hydride generator HGX-100 (CETAC Technologies Inc., Omaha, Nebraska, USA).

The high-performance liquid chromatography system consisted of a Hewlett Packard quaternary HPLC pump series 1100 (Hewlett Packard, Waldbronn, Germany) and a Rheodyne 6-port injection valve with a 50- μ l injection loop. The separations were performed on a Hamilton (Reno, USA) PRP-X100 anion-exchange column (25 cm x 4.1 mm i.d., spherical 10- μ m styrene-divinylbenzene particles with trimethylammonium exchange sites). The mobile phase was pumped through the analytical column at a flow rate of 1.5 ml min⁻¹. The chromatographic system was connected with the Hewlett Packard HP 4500 ICP-MS with a 80-cm PEEK (polyether ether ketone) capillary tubing (1/16-in i.d.) that connected the HPLC column outlet to the inlet of the Babington nebulizer of the ICP-MS. The ion intensities at *m/z* 82 (⁸²Se) and 77 (⁷⁷Se) were monitored using the "time-resolved mode". The integration time was set to 0.3 s.

During instrumental optimisation a standard solution of selenite (50 Se μ g l⁻¹) in the applied mobile phase was continuously pumped to the ICP-MS at a flow rate of 1.5 ml min⁻¹. The tuning parameters for the ICP-MS were set with respect to highest signal intensities at *m/z* 82 and lowest background at *m/z* 89.

The chromatographic peaks resulting from the coupled HPLC-ICP-MS system were recorded in the graphics mode i.e., the signal intensity in counts was recorded. The peak areas were determined using the data analysis program of the MSD ChemStation software (G1701AA, Version A.03.00, Hewlett Packard). The selenium compounds were quantified with external calibration curves established with each of the selenium compounds.

2.2 Reagents, standards, and mobile phases for HPLC-ICP-MS

All commercial chemicals were used without further purification. Sodium selenate (p.a., 71947) and seleno-DL-methionine (Biochemie, 84925) were purchased from Fluka, selenium dioxide (SeO₂ in diluted nitric acid, 1000 mg Se l⁻¹, Titrisol, 9915) from Merck, and seleno-DL-cystine (laboratory grade, S-1650) from Sigma. Stock solutions were prepared with Milli-Q water (18.2 MW cm) from anhydrous sodium selenate (1196.4 mg to 500 ml, 1000 mg Se l⁻¹), from selenomethionine (24.8 mg to 20 ml, 500 mg Se l⁻¹), and from selenocystine (5.3 mg to 20 ml, 125 mg Se l⁻¹). The stock solutions were stored in the refrigerator at -20 °C before use.

Solutions containing each of the four selenium compounds with concentrations in the range 5–50 μ g Se l⁻¹ were prepared by appropriate dilution of the stock solutions with Milli-Q water.

The mobile phase for the anion-exchange HPLC was prepared by dissolving 2.10 g (10 mM), citric acid monohydrate (puriss p.a., Fluka, 27490) in Milli-Q water, adjusting the pH of this solution to 5.0 by addition of NH₃ (suprapure, Merck, 5428), and filling to 1000 ml. To the citric acid buffer 2 % methanol (puriss p.a., Fluka, 65543) was added.

Calibration curves for the HPLC-ICP-MS measurements of the natural samples were obtained by injecting chromatographing aliquots (50 μ l) of solutions containing 5.00, 10.0, 20.0 or 50.0 μ g Se l⁻¹ of selenocystine, selenite, selenomethionine and selenate.

As extraction solvents Milli-Q water, citric acid, formic acid (Merck, suprapure, 11670), or sodium hydroxide (puriss p.a., Fluka, 71690) were used.

The citrate/phosphate buffer used for enzymatic hydrolysis was prepared by dissolving 21.0 g citric acid monohydrate and 11.5 g NH₄H₂PO₄ (puriss p.a., Fluka 7908) in water, adjusting the pH to 7.3 with NH₃, and filling to 1000 ml.

A 0.1 M NaOH solution was prepared by dissolving 4 g NaOH and filling to 1000 ml.

2.3 Reagents for the determination of total selenite by HG-ICP-MS

All solutions were prepared with Milli-Q water. Concentrated hydrochloric acid (Merck p.a., 319) was purified in

an MLS Duopur quartz subboiling distillation unit. Sodium hydroxide and sodium borohydride were of analytical grade (Fluka puriss p.a., 71960, Merck p.a., 6371). Standard solutions for selenium in the range of 5.0 to 50.0 $\mu\text{g l}^{-1}$ were prepared by appropriate dilution of the standard stock solution (SeO_2 in diluted nitric acid, 1000 mg Se l^{-1} , Merck, Titrisol, 9915) with 1.0 M HCl.

2.4 Extraction procedures

Powdered commercially available feeds, to which selenium had been added as sodium selenite, were obtained from the Federal Office and Research Centre for Agriculture, Vienna, Austria.

Aliquots (~ 0.2 g) of the powdered feeds were weighed to 0.1 mg into 50-ml polyethylene tubes. Milli-Q water (10 ml), 0.1 M NaOH, 0.1 M, 0.01 M or 0.001 M citric acid was added. The tubes were shaken for 14 hours. The sodium hydroxide extracts were shaken for 4 hours. The mixtures were centrifuged at 3000 rpm. All extracts were filtered through 0.22- μm Millex-GS cellulose ester filters (Millipore, Bedford, USA) and chromatographed on the HPLC-ICP-MS system.

Enzymatic hydrolysis: Aliquots (0.2 g) of the feeds were weighed to 0.1 mg into 50 ml polyethylene tubes together with 20 mg protease (pronase, Sigma, P-5147) and either 10 ml Milli-Q water or 10 ml 0.1 M citrate/phosphate buffer. The tubes were shaken at 37 °C in a water bath for 4 hours. After centrifugation and filtering the extracts were chromatographed on the HPLC-ICP-MS system.

2.5 Quantification of selenite in feed extracts by HG-ICP-MS

Aliquots (~ 1.0 g) of powdered feeds were weighed to 0.1 mg into 50-ml polyethylene tubes. Milli-Q water (50 ml) was added. The tubes were shaken for 14 hours. The mixtures were centrifuged at 3000 rpm. All extracts were filtered through 0.22- μm Millex-GS cellulose ester filters. 20 ml of the extract were spiked with 50 $\mu\text{g l}^{-1}$ selenite and to 20 ml of the same extract additionally 0.2 ml of concentrated HCl was added. The concentration of selenite was determined in these solutions by HG-ICP-MS with an external calibration curve established with selenium standard solutions.

3. Results and discussion

3.1 Separation technique

Anion-exchange HPLC was chosen, because in artificial feeds selenite is the most probable compound to be found. Furthermore, ion exchange columns allow the use of largely aqueous mobile phases. High amounts of organic solvent is known to destabilise or extinguish the argon plasma of the ICP-MS (PEDERSEN and LARSEN, 1997). To prevent salt build-up on the sampler and skimmer cones the chromatographic mobile phase should contain salts with organic anions, preferably such as acetates, formiates, or citrates.

In this work a citrate buffer was used as mobile phase. Citric acid is a tricarboxylic acid, all three pK -values are in the acidic pH range ($\text{pK}_1 = 3.14$, $\text{pK}_2 = 4.77$, $\text{pK}_3 = 6.39$) (MARTELL and SMITH, 1977). At pH 8 the acid is fully deprotonated.

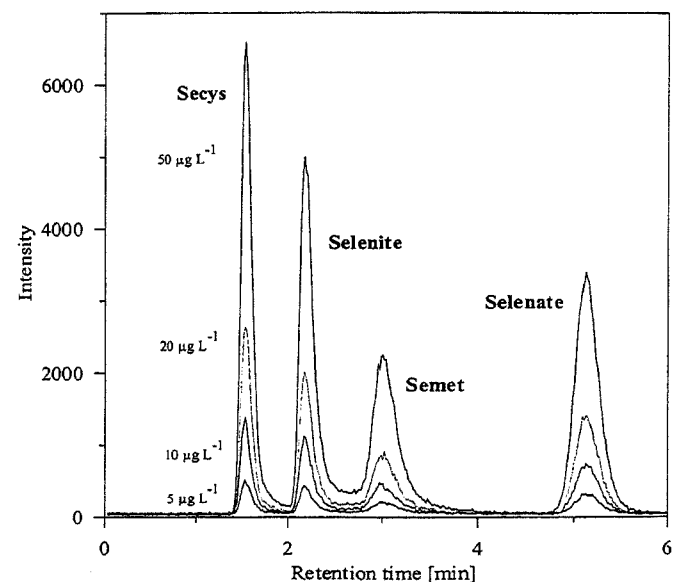


Figure 1: Standard chromatograms obtained for selenocystine, selenite, selenomethionine, and selenate, concentration of selenium compounds: 5, 10, 20, 50 ng ml^{-1} each (Hamilton PRP-X100 column, 50 μl injected, 10 mM citric acid pH 5.0, 2% methanol as mobile phase; flow rate of 1.5 ml min^{-1} ; ICP-MS as selenium specific detector, m/z 82)

Abbildung 1: Chromatogramme erhalten aus einem Gemisch der Reinsubstanzen Selenocystin, Selenit, Selenomethionin und Selenat, bei Konzentrationen der Seleniumverbindungen von je 5, 10, 20, 50 ng ml^{-1} (Hamilton PRP-X100 Säule, 50 μl eingespritzt, 10 mM Zitronensäure pH 5.0, 2% Methanol als Mobile Phase; Flußrate 1.5 ml min^{-1} ; ICP-MS als selenspezifischer Detektor, m/z 82)

Previous investigations using aqueous extracts of feeds showed that only selenite and sometimes small signals of selenate could be identified in these samples. Therefore a polymer-based PRP-X100 anion-exchange column (Hamilton) was used for separation of selenium compounds in feeds.

The Hamilton PRP-X100 strong anion-exchange column has strongly basic quaternary ammonium groups as exchange sites, which are bound to the polymeric stationary phase. Negatively charged compounds, such as selenous acid and selenic acid, interact with the quaternary ammonium sites.

The ideal result of any separation is baseline separation of all compounds under investigation with reasonably short retention times to obtain sharp signals and keep the measurement time low. In this work, calibration curves for selenite, selenate, selenomethionine, and selenocystine were made. With a mobile phase containing 10 mM citric acid (pH 5) and 2 % methanol the four selenium compounds were separated within 6 min (Figure 1).

3.2 Determination of selenium in feeds with HPLC-ICP-MS

Varying extraction solvents were investigated with respect to the extraction efficiency of selenium compounds from feeds supplemented with sodium selenite. In principle, water should be the solvent of choice, because the selenite

present in feeds is expected to be fully soluble. The solubility of selenium compounds in water determines the bio-availability of the selenium compounds to a large extent.

Therefore, different feeds were investigated with respect to the water-extractable selenium compared to total selenium, that had been determined with Hydride-Generation-ICP-MS (Table 1). In Figure 2 some chromatograms of aqueous feed extracts are shown. As expected, mainly selenite is extractable. The chromatograms of some of the extracts exhibit a small selenate signal, too. However, the concentration of selenite found using HPLC-ICP-MS does not match the total concentration of selenium in the samples. The concentration of total selenium in the feeds investigated differs considerably from the concentration of selenite found in the water-extracts. Only 15 to 50 % of the total selenium concentration was identified as selenite using HPLC-ICP-MS.

The low recovery rate of selenite in aqueous extracts of feeds was unexpected, as it was expected to be fully water soluble. To investigate, whether precipitates of metal selenites, such as $\text{Fe}_2(\text{SeO}_3)_3$, CuSeO_3 or PbSeO_3 , are formed in the feeds, extraction was performed with acidic solvents. Dilute acids dissolve all metal selenites, thus, the concentration of selenite is expected to increase. Concentrations of citric acid ranging from 0.001 M to 0.1 M were tested as extraction solvent (Figure 3). Results in fact showed that the concentration of selenite in the extracts decreased with decreasing pH. No difference in the extractable selenite was observed using 0.001 and 0.01 M citric acid compared to

Table 1: Determination of selenite and selenate in water extracts of feeds. Comparison with total selenium concentration determined with HG-ICP-MS. Experimental parameters for HPLC-ICP-MS: 10 mM citric acid pH 5, 2% methanol as mobile phase, 50 μl injected, flow rate of 1.5 ml min^{-1} , ICP-MS as selenium specific detector, m/z 82

Tabelle 1: Bestimmung von Selenit und Selenat in Wasserextrakten von Futtermitteln. Vergleich mit den mit Hydridentwicklungs-ICP-MS bestimmten Gesamtgehalten. Experimentelle Parameter für die HPLC-ICP-MS: 10 mM Zitronensäure pH 5, 2 % Methanol als mobile Phase, 50 μl aufgegeben, Flußrate 1,5 ml pro Minute, ICP-MS als Selen-spezifischer Detektor auf $m/z = 82$

No	Compound feed for	HG-ICP-MS			HPLC-ICP-MS		
		Total selenium [$\mu\text{g kg}^{-1}$]			Selenite [$\mu\text{g kg}^{-1}$]	Selenate [$\mu\text{g kg}^{-1}$]	
1	Cattle for fattening	860	± 80	(3)	280	± 140	(3)
2	Cattle for fattening	990	± 70	(3)	280	± 40	(3)
3	Turkeys	520	± 30	(3)	70	± 7	(3)
4	Pigs	1900	± 170	(3)	590	± 70	(3)
5	Sows	3930	± 460	(3)	1100	± 150	(3)
6	Laying hens	930	± 90	(3)	210	± 20	(3)
7	Piglets	910	± 50	(3)	350	± 140	(3)
8	Piglets	720	± 30	(3)	122	± 5	(3)
9	Pigs	3700	± 80	(3)	1140	± 220	(3)
10	Pigs for fattening	820	± 80	(3)	300	± 25	(3)
11	Piglets	1270	± 130	(3)	660	± 90	(3)

Number of replicates in parenthesis

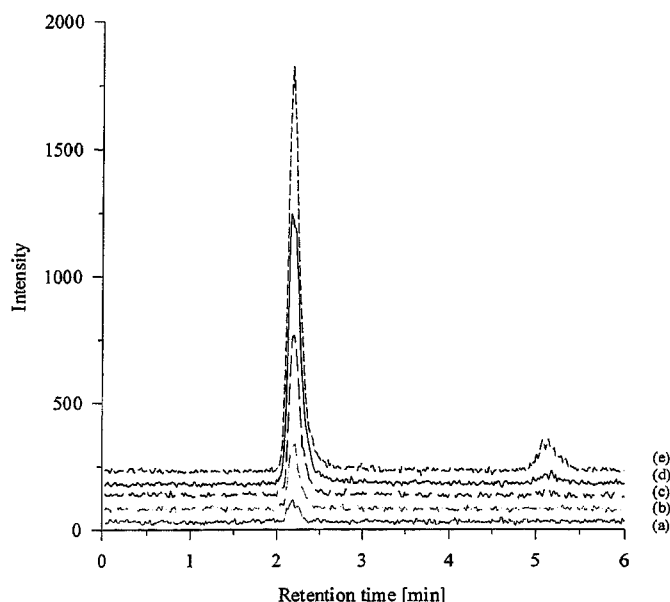


Figure 2: Determination of selenite and selenate in water extracts of (a) feed 3, (b) feed 8, (c) feed 2, (d) feed 1, (e) feed 4 (dilution factor ~ 40 , other experimental parameters see Fig. 1)

Abbildung 2: Bestimmung von Selenit und Selenat in Wasserextraktion von (a) Futtermittel 3, (b) Futtermittel 8, (c) Futtermittel 2, (d) Futtermittel 1, (e) Futtermittel 4 (Verdünnungsfaktor ~ 40 , andere experimentelle Parameter siehe Abb. 1)

aqueous extracts. With 0.1 M citric acid, however, the chromatographic signal for selenite was very small. Similar results were obtained, when selenite was extracted from feeds with formic acid. A lot of compounds are known to exist in artificial animal diets, which may be co-extracted with selenite under acidic conditions, such that the analytical column can easily be overloaded. Additionally, compounds, which are soluble in dilute acids can precipitate in the column at the eluent buffer pH of 5.0. It was therefore necessary to be ascertained whether the small selenite signal obtained from the extraction with 0.1 M citric acid corresponded to the actual concentration in the extract. This required the concentrations of selenite in the aqueous and acidic extracts to be evaluated not only using an external calibration, but also using a standard addition method. The selenite concentrations of the water extracts obtained with both calibration methods were found to be in the same order of magnitude ($\sim 300 \mu\text{g Se kg}^{-1}$). Less than 30 % of the selenite in the aqueous extract was found in the acid extract by the external calibration method. Standard addition to the extract, however, showed a recovery of about 80 % of the added selenite.

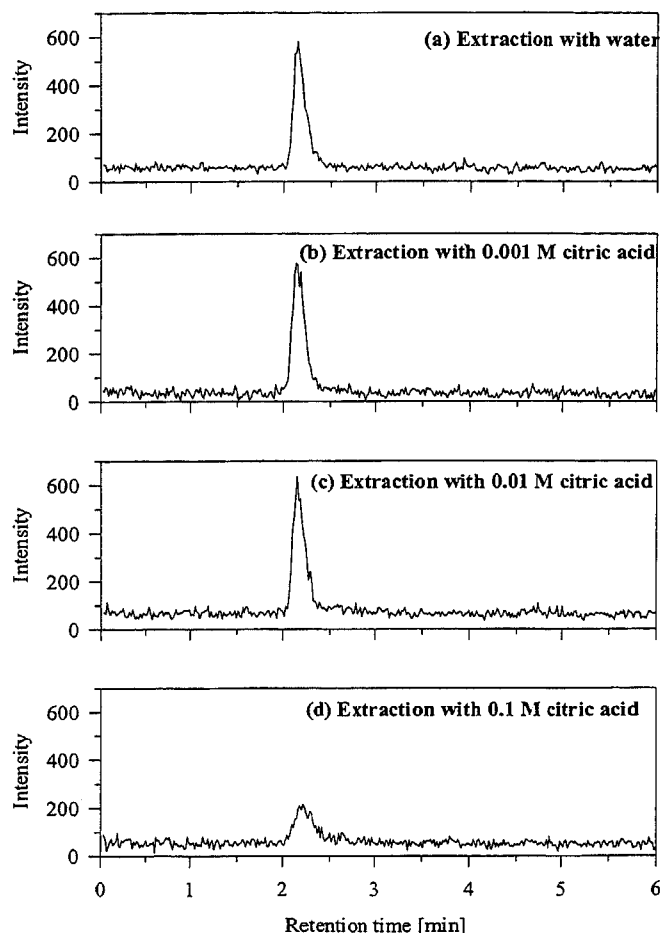


Figure 3: Determination of selenite in extracts of feed No. 10; extraction solvent (a) water, (b) 0.001 M citric acid, (c) 0.01 M citric acid, (d) 0.1 M citric acid (other experimental parameters see Fig. 1)

Abbildung 3: Bestimmung von Selenit in Extrakten von Futtermittel 10 mit den Extraktionsmitteln (a) Wasser, (b) 0,001 M Zitronensäure, (c) 0,01 M Zitronensäure, (d) 0,1 M Zitronensäure (andere experimentelle Parameter siehe Abb. 1)

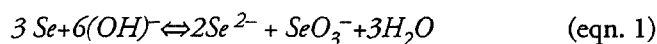
Obviously, water is the most promising extraction solvent for these animal feeds, because the concentration of co-extracted non-selenium compounds is smaller than with acid extraction.

Furthermore, an experiment was carried out to prove, if other selenium compounds possibly incorporated into proteins could be released with an enzymatic extraction technique. A non-specific protease (pronase), which had been isolated from *Streptomyces griseus*, was chosen as the enzyme. Using an unspecific protease it is possible to break the peptidic bonds of any protein present in biological materials. For enzymatic hydrolysis either a buffer solution (0.1 M citrate/phosphate pH 7.3) (OLIVAS et al., 1996; GILON et al.,

1996) or pure water (BIRD et al., 1997) were used as extraction solvents. But in the extracts no seleno amino acids could be found with HPLC-ICP-MS. The chromatograms of the enzymatic extracts did not differ from the one of the pure water extracts. As the enzymatic hydrolysis of the feeds did not result in a release of any other selenium compound, the selenium, which is not extractable with water, is obviously not incorporated into proteins as selenoamino acids.

These results show that a large part of the supplemented selenite does not seem to be biologically available; probably as it is converted into less soluble forms.

From literature, it was found that the addition of selenite to reducing soil could lead to the formation of elemental selenium (TOKUNAGA et al., 1996). The aqueous extracts of the feeds have reducing properties, and it is possible that a reduction process could have taken place. Selenites are known to be converted easily to elemental selenium by many reducing agents. The reduction process is kinetically favoured especially in acidic solution. In alkaline solutions the reduction is kinetically hindered. Additionally, basic solutions may dissolve already formed elemental selenium (eqn. 1).



Therefore, the selenite concentration was expected to increase in alkaline extracts of feeds compared to aqueous extracts. The results for some feeds are shown in Figure 4. In these chromatograms it can be observed that the selenite concentration found in NaOH extracts raised by more than 100 %.

The reduction of selenite to elemental selenium is kinetically controlled. A time-dependent decrease in the concentration of selenite is expected. Additionally, acidic conditions are expected to favour the formation of elemental selenium from selenite.

It was therefore necessary to investigate, whether the concentration of selenium in water extracts of feeds decreases with time or not. Two feeds were therefore extracted with water, 50 µg l⁻¹ selenite was added and the concentration of selenium was determined 2 days, 8 days, and 24 days after extraction by HG-ICP-MS. The extracts were stored in the refrigerator. The effect of acidity on the decrease of the concentration of selenite was also studied.

In Figure 5 the dependence of the selenite concentration on the storage time of water extracts, which were spiked with 50 µg l⁻¹ selenite, is shown. A notable decrease in the concentration of selenite with time can be observed. Further-

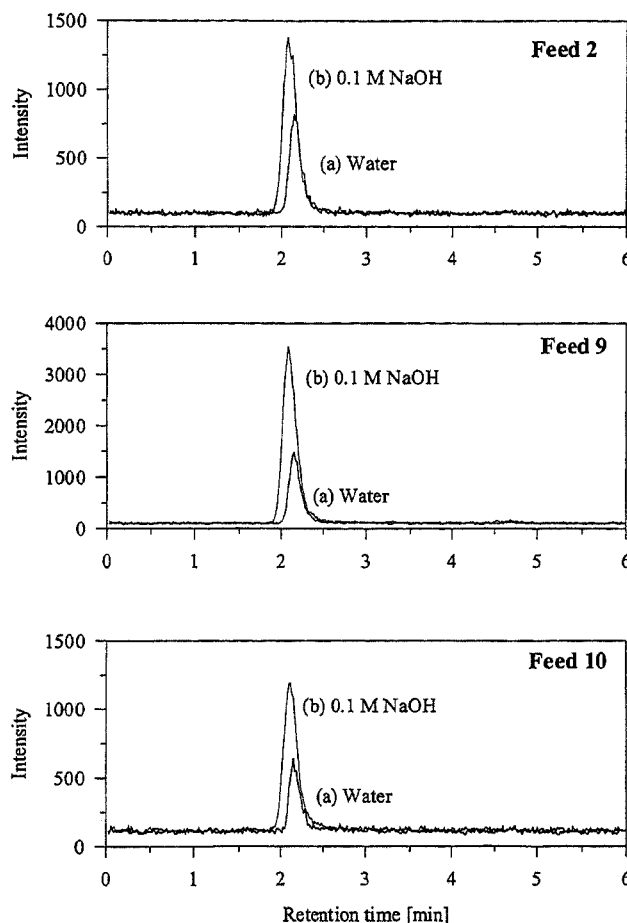


Figure 4: Determination of selenite in the extracts of feed No. 2, 9, and 10; extraction solvent (a) water, (b) 0.1 M NaOH (other experimental parameters see Fig. 1)

Abbildung 4: Bestimmung von Selenit in den Extrakten von Futtermittel 2, 9 und 10 mit dem Extraktionsmittel (a) Wasser, (b) 0,1M NaOH (andere experimentelle Parameter siehe Abb. 1)

more, it can be seen in this figure that the concentration of selenite decreases to a larger extent, when 0.1 M HCl is added to the extracts. These results show that selenite in feed extracts is obviously converted to probably elemental selenium with increasing storage time by reaction with co-extracted reductants, like reducing sugars, ascorbic acid, etc.

4. Conclusion

The matrix of artificial animal feeds is of important consideration in the determination of total selenium following mineralization of the feed source. In this work, it was anticipated that a recovery rate of supplemented soluble selenite

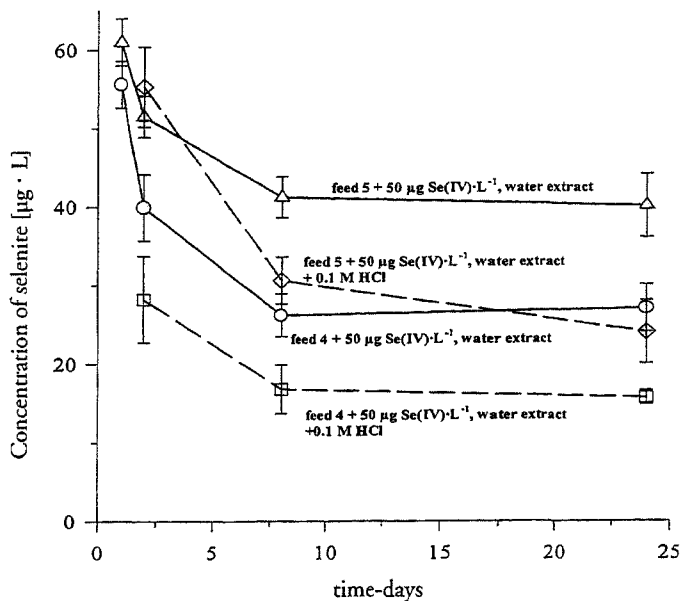


Figure 5: Dependence of the selenite concentration on the storage time of water extracts of feeds, which were spiked with $50 \mu\text{g l}^{-1}$ selenite, determined with HG-ICP-MS

Abbildung 5: Abhängigkeit der Selenitkonzentration von der Lagerzeit der Wasserextrakte der Futtermittel bei Zusatz von $50 \mu\text{g l}^{-1}$ Selenit, bestimmt mit HG-ICP-MS

in the aqueous extracts of animal feeds of between 90 to 100 % was achievable. However, the actual recovery rates in the feeds were found to range from between 15 and 55 %, using an aqueous extraction. It is thought that part of the selenite is reduced to elemental selenium during extraction. The determination of selenite in the feeds used by HPLC of aqueous extracts is more reliable than the determination of total selenium after complete mineralization of the sample.

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