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Determination of trace amounts of selenium in poultry feedstuffs by gas chromatography

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Abstract

In view of the importance of establishing reliable selenium concentration levels in different kinds of feedstuffs, the purpose of this work was to develop optimum experimental conditions for the isolation and GC determination of selenium as its chelate with 4-nitro-1,2-diaminobenzene. It was shown that ignition of the sample in an oxygen flask followed by reduction of Se(VI) to Se(IV) and the formation of 5-nitro-2,1,3-benzoselenadiazole chelate in HCl medium is a relatively rapid procedure giving a low blank value and allowing the determination of selenium in commercial feedstuffs and similar biological samples. The method was validated by the analysis of suitable certified or standard reference materials.

1. Introduction

Until 1957, selenium was known only as one of the most toxic elements. In that year, Schwarz and Foltz [1] identified selenium as a constituent of cellular glutathione peroxidase (GSH-Px) and since then its presence in enzymes such as plasma GSH-Px, phospholipid hydroperoxide GSH-Px, I iodothyronine-5′-diiodinase and selenoprotein P has been established, thus providing evidence for the involvement of selenium in numerous metabolic processes [2–4].

Symptoms of Se deficiency in poultry are manifested as exudative diathesis, fibrosis of the pancreas, fibrosis of the skeletal musculature and muscular dystrophy, whereas permanent excessive doses of selenium in poultry feed can cause blind staggers, alkali disease and acute toxicity, which are manifested in a decrease in egg laying and flying capability, in different anomalies of the embryo, incidence of paralysis and limping, liver cirrhosis, loss of feathers, etc. [5–7].

According to the Enviromental Health Criteria [8], the daily requirement of selenium in poultry is 30–50 $\mu g\ kg^{-1}$, provided that the amount of vitamin E in the daily ration is adequate. The major part is provided by grains, which are the main component of their feedstuffs and can contain, according to literature data [9], very variable amounts of selenium.

The legal regulation for the quality of different feeds in Slovenia specifies a minimum content of 150 $\mu g\ kg^{-1}$ of Se for dry poultry feedstuffs when added in the form of sodium selenite or selenate. The corresponding regulation for the maximum allowed amounts of harmful constituents in dry poultry feedstuffs permits a limiting value of 500 $\mu g\ kg^{-1}$ of Se. In spite of

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this, official control methods for selenium are lacking. Also, reliable data on the concentration levels of selenium in natural or industrially prepared feedstuff mixtures are very scarce or do not exist.

For the determination of microgram and nanogram amounts of selenium in biological samples the most often used and sensitive methods (with limits of detection from 0.2 to 50 $\mu g\ kg^{-1}$) are fluorimetry, hydride generation atomic absorption spectrometry, radiochemical neutron activation analysis (RNAA), instrumental NAA and GC with electron-capture detection (ECD) [6,10,11]. Each of these methods has advantages and also disadvantages such as matrix effects, interferences, contamination problems and reagent purity. In spite of the minimum possibilities of sample contamination and of the sensitivity of the RNAA method [12–18], our experience shows that this method is time consuming and too expensive for routine work.

For the determination of such amounts of selenium, the GC method seems to be very attractive [19–23]. In addition to the availability of such equipment in our laboratory, the motives for developing this technique for the determination of selenium in feeds and food products were to reduce costs, obtain rapid and accurate results, and not least to exploit the possibility of checking the results with those obtained for the same samples by RNAA. This required investigation of the optimum experimental conditions for the isolation and GC determination of selenium in the form of its chelate with 4-nitro-1,2-diaminobenzene from HCl medium, in combination with a simple destruction technique giving high recoveries and low blank values. This latter is very important when low concentrations of selenium must be determined. Although the prescribed Se concentrations in feedstuffs, in the range 150–500 $\mu g\ kg^{-1}$, are relatively high, a margin of sensitivity is required and when checking individual components of feeds, such as cereals growing in selenium-deficient areas [6], the levels may be very low (few $\mu g\ kg^{-1}$ range). Oxygen combustion in a closed flask was shown to fulfil these requirements.

The reliability of the method and the results obtained were checked by the analysis of appropriate certified or standard reference materials. Further, a selection of local feedstuffs were analysed and compliance with the regulations was checked.

2. Experimental

2.1. Sample preparation

Samples of mixed commercial poultry feedstuffs with declared supplements of selenium (150 $\mu g\ kg^{-1}$) were homogenized according to a standard method for the preparation of laboratory samples for analytical purposes [24] and ground.

2.2. Reagents

All reagents were of analytical grade or better, namely anhydrous Na_2SeO_4 (Sigma, Deisenhofen, Germany), 4-nitro-1,2-diamino-benzene (4NDB), 30% HCl (Suprapur), $Mg(NO_3)_2$ and urea[$CO(NH_2)_2$] (Merck, Darmstadt, Germany), toluene(Nanograde) (Promochem, Wesel, Germany) and Whatman No. 42 filterpaper (Whatman, Maidstone, UK) for lining the combustion boats.

2.3. Preparation of standard Se solution

The synthesis of 5-nitro-2,1,3-benzoselenadiazole chelate (5NBSeD) from Na_2SeO_4 and 4NDB was performed according to McCarthy *et al.* [21]. Its purity was checked by the determination of its melting point (224–226°C) and by elemental analysis (found, C 31.20, H 1.22, N 18.20; theoretical values, C 31.59, H 1.33, N 18.40%) and also by mass spectrometry (m/z = 229).

For the preparation of a stock standard solution, 1.271 mg of 5NBSeD chelate were weighed and transferred quantitatively with toluene into a 50-ml volumetric flask. A suitable aliquot of this solution, which is stable for more than 1 month, was diluted every 14 days with toluene to obtain working solutions with selenium concentrations

of 5.28, 10.56, 15.84 and 21.12 ng ml^{-1}. The calibration graphs for the standard solutions correlated adequately ($r = 0.998$).

2.4. Destruction of samples and isolation of selenium

Two methods of sample destruction were tested, oxygen combustion and destruction with $Mg(NO_3)_2$, followed by isolation of the Se chelate. The procedure is given in more detail in Fig. 1.

2.5. Recovery of the procedure for GC determination of Se

The recovery of the whole procedure for the GC determination of Se (with oxygen combustion) was obtained by standard additions of known amounts of Se (20–200 ng) via the Se stock solution to the filter-paper and also to some real samples.

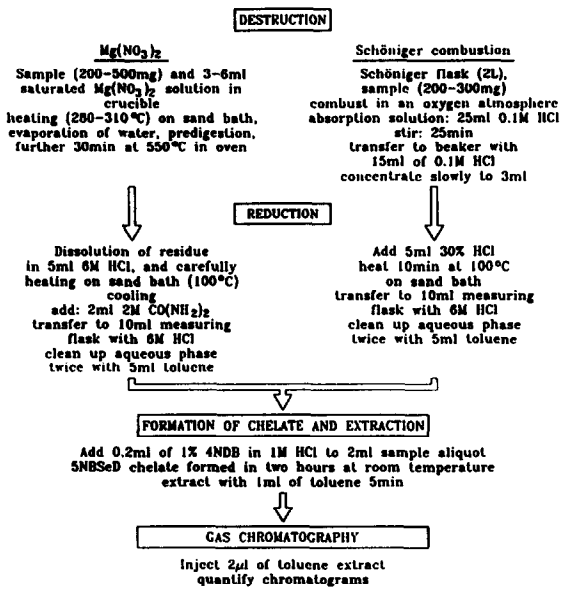

Fig. 1. Scheme for determination of selenium in feedstuffs by GC.

2.6. Apparatus and working conditions

A Hewlett-Packard (Avondale, PA, USA) Model 5890A gas chromatograph with a ^{63}Ni electron-capture detector was used for selenium determination. The temperatures were: column 200°C, injector 260°C and detector 280°C. The carrier gas was nitrogen (99.995%; Ruše, Slovenia) at a flow-rate of 40 ml min^{-1}. An HP 19094A-A28 2.35 m × 0.2 cm I.D. glass column (Hewlett-Packard) packed in our laboratory with 3% OV-17 stationary phase on Chromosorb W HP (0.15–0.20 mm) (Serva, Heidelberg, Germany) was used for the separation of 5-NBSeD. The sensitivity of the recorder was 1 or 10 mV with a charge speed of 0.63 cm min^{-1}, according to the concentration levels.

3. Results and discussion

Samples of plant origin normally contain selenium at the µg kg^{-1} level. For the determination of such concentrations all analytical methods require prior destruction of the sample.

First, the decomposition of samples with a saturated solution of $Mg(NO_3)_2$ was studied. The advantages of saturated $Mg(NO_3)_2$ solution as a destructive agent are particularly its ease of manipulation, the need for only simple equipment, its suitability for the destruction of both fresh and dry samples and the very small losses of selenium (about 6%, as found by the ^{75}Se tracer technique), in spite of heating the residue at 550°C. However, the RNAA results for the determination of selenium in the saturated $Mg(NO_3)_2$ solution used showed high blank values (30–50 µg kg^{-1}), and the volume necessary for complete destruction of fresh or lyophilized samples was 3–6 ml (depending on the sample mass). A further disadvantage of this destruction technique was the appearance of some unindentified interfering peaks in the chromatograms of the Se chelate, in spite of adding 2 ml of 2 *M* $CO(NH_2)_2$ after reduction of Se(VI) to Se(IV) and cleaning up the toluene extract of

the Se chelate with 6 *M* HCl. Therefore, we decided that this destruction procedure was not practicable for use in combination with GC analysis.

From our previous experience [18] and the above facts, a simple combustion procedure in an oxygen atmosphere seemed to be the most promising in combination with the GC determination of Se. Provided that the concentration of Se in the filter-paper is below 15 $\mu g\ kg^{-1}$ (as was the case with the Whatman No. 42 filter-paper), then with the use of only a small volume of Suprapur 30% HCl as adsorption solution and for reduction of Se(VI) to Se(IV) (6 ml), the total blank value arising from the filter-paper, HCl and 4NDP reagent is minimal. Chromatograms of the solvent, Se standard, 6 *M* HCl, the reagent blank and a real sample are shown in Fig. 2.

The method was tested by the use of the standard addition technique, performed using filter-paper alone and with various matrices carried through the whole procedure, and a mean overall recovery of 88.1 ± 7.8% was routinely obtained.

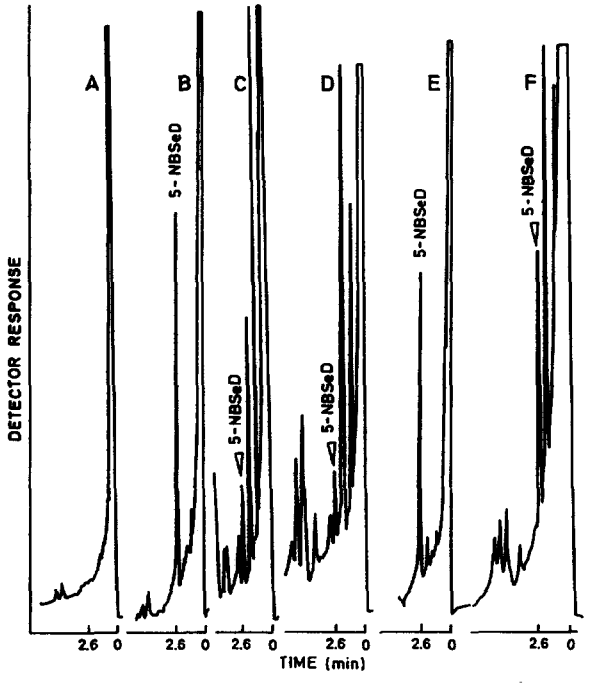

Fig. 2. Chromatograms for (A) solvent, (B) 10.56 pg Se standard, (C) 2.4 pg Se in reagent blank, (D) 2.1 pg Se in 6 *M* HCl, (E) 21.12 pg Se standard and (F) 17.79 pg Se in feedstuff Bro-val 1.

Using the described method, some commercial poultry feedstuffs were analysed. From the results obtained (Table 1), it is evident that selenium levels are in the range required by the Slovenian regulation for poultry feedstuffs (150–500 $\mu g\ kg^{-1}$).

The sample signal from the chromatogram may be quantified either by immediate injection into the column of a suitable aliquot of standard solution of 5NBSeD in toluene, or from a calibration graph constructed daily. It was found that the response of the detector was constant throughout the day for the same amount of Se chelate.

The reliability of the results was checked by the analysis of appropriate certified or standard reference materials and by comparison with results obtained by RNAA in our laboratory. From the results obtained (Tables 2 and 3), good agreement with both the certified and RNAA values is evident.

The advantageous features of the method, namely the simple and rapid destruction procedure (combustion in oxygen), the low blank value (the contributions only of the filter-paper and Suprapur hydrochloric acid), the non-requirement for a clean-up procedure of the

Table 1
Results ($\mu g\ kg^{-1}$ dry mass) for selenium in commercial poultry feedstuffs marketed in Slovenia

Commercial poultry feedstuff	$\bar{x} \pm s$ $(n)^a$
PŠ	276 ± 22 (6)
Bro-val 1	215 ± 22 (6)
Bro-jr	250 ± 33 (6)
Bro-val 2	275 ± 31 (5)
Jr	239 ± 21 (6)
NSK finely ground	264 ± 16 (6)
NSK-2	238 ± 9 (6)
Bro-fin	271 ± 15 (6)

[a] Mean ± standard deviation; n = number of determinations.

Table 2
Results for selenium in certified or standard reference materials ($\mu g\ kg^{-1}$ dry mass)

Sample	$\bar{x} \pm s$ $(n)^a$	Certified value
Lucerne 1992 International Analytical Group [25]	105 ± 13 (4)	117 ± 12
Mixed feed 1992 International Analytical Group [25]	432 ± 16 (4)	420 ± 50
BCR CRM 189 Wholemeal Flour	125 ± 9 (4)	132 ± 10
NIST SRM 1568 Rice Flour	362 ± 25 (4)	400 ± 100
NIST SRM 1567a Wheat Flour	973 ± 27 (4)	1100 ± 200

[a] Mean ± standard deviation; n = number of determinations.

Table 3
Comparison of results ($\mu g\ kg^{-1}$ dry mass) for selenium in some poultry feedstuffs and BCR CRM 189 Wholemeal Flour determined by GC and RNAA

Sample	$\bar{x} \pm s$ $(n)^a$	
	GC	RNAA
PŠ	276 ± 22 (6)	282 ± 26 (6)
Bro-val	215 ± 22 (6)	236 ± 18 (6)
KIS Feedstuff	291 ± 7 (4)	320 ± 28 (6)
BCR CRM 189 Wholemeal Flour[b]	125 ± 9 (4)	137 ± 10 (3)

[a] Mean ± standard deviation; n = number of determinations.
[b] Certified Se content: 132 ± 10 $\mu g\ kg^{-1}$.

5NBSeD extract in toluene, the high recovery of 88.1 ± 7.8% (determined by standard additions) and the low detection limit (1.2 $\mu g\ kg^{-1}$) make it suitable and convenient for the routine determination of selenium in poultry feedstuffs.

4. References

[1] K. Schwarz and C.M. Foltz, *J. Am. Chem. Soc.*, 39 (1957) 3239.
[2] D. Behne, A. Kyriakopoulos, H. Meinhold and J. Kohrle, *Biochem. Biophys. Res. Commun.*, 173 (1990) 1143.
[3] B.A. Zachara, *J. Trace Elem. Electrolytes Health Dis.*, 6 (1992) 137.
[4] J. Arthur, F. Nicol and J.C. Beckett, *Am. J. Clin. Nutr., Suppl*, 57 (1993) 236S.
[5] *Selenium in Nutrition*, National Academic Press, Washington, DC, revised ed. 1983, p. 174.
[6] J.F. Combs and S.B. Combs, *The Role of Selenium in Nutrition*, Academic Press, New York, 1986, p. 532.
[7] L.R. McDowel, *Minerals in Animal and Human Nutrition*, Academic Press, Orlando, FL, 1992, p. 524.
[8] *Environmental Health Criteria 58, Selenium*, WHO, Geneva, 1987, p. 306.
[9] R. Seeger and H.G. Neuman, *Dtsch. Apoth.-Ztg.*, 131 (1991) 354.
[10] S.E. Raptis, G. Kaiser and G. Tölg, *Fresenius' Z. Anal. Chem.*, 316 (1983) 105.
[11] J. Piwonka, G. Kaiser and G. Tölg, *Fresenius' Z. Anal. Chem.*, 321 (1985) 225.
[12] A.R. Byrne and L. Kosta, *Talanta*, 21 (1974) 1083.
[13] H. Polkowska-Motrenko, M. Dermelj, A.R. Byrne, A. Fajgelj, P. Stegnar and L. Kosta, *Radiochem. Radioanal. Lett.*, 53 (1982) 319.
[14] M. Dermelj, G. Hancman, A. Gosar, A.R. Byrne and L. Kosta, *Vestn. Slov. Kem. Druš.*, 23 (1985) 127.
[15] M. Dermelj, A.R. Byrne, M. Franko, B. Smodiš and P. Stegnar, *J. Radioanal. Nucl. Chem., Lett.*, 106 (1986) 91.
[16] M. Dermelj, M. Horvat, A.R. Byrne and P. Stegnar, *Chemosphere*, 16 (1987) 877.
[17] A. Fajgelj, M. Dermelj, A.R. Byrne and P. Stegnar, *J. Radioanal. Nucl. Chem. Lett.*, 128 (1988) 93.
[18] M. Dermelj, V. Stibilj, J.M. Stekar and A.R. Byrne, *Fresenius' J. Anal. Chem.*, 340 (1991) 258.
[19] C.F. Poole, N.J. Evans and D.C. Wibberley, *J. Chromatogr.*, 136 (1977) 73.
[20] K. Toei and Y. Shimoishi, *Talanta*, 28 (1981) 967.
[21] T.P. McCarthy, B. Brodie, J.A. Milner and R.F. Bevill, *J. Chromatogr.*, 225 (1981) 9.
[22] K.W.M. Siu and S.S. Berman, *Talanta*, 31 (1984) 1010.
[23] K. Johansson and A. Olin, *J. Chromatogr.*, 589 (1992) 105.
[24] *Methodenbuch, Band III, Die Chemische Untersuchung von Futtermitteln*, Neumann, Neudamm, Germany, 1976.
[25] *Ringtest of the International Analytical Group 1992*, Bedrijfslaboratorium voor Grond- en Gewasonderzoek, International Analytical Group, Oosterbeek, Netherlands, 1992, p. 18.