

The Determination of Selenium in Animal Feeding Stuffs

J. H. HAMENCE AND D. TAYLOR

Dr Bernard Dyer & Partners (1948) Ltd, 20 Eastcheap, London E.C.3

A method is described for determination of low levels of selenium, which are required to be declared by the Feeding Stuffs Regulations of 1976, and which is based on wet ashing the finely ground sample followed by conversion to 5-nitropiazselenol which is detected by gas chromatography using an electron capture detector.

The Feeding Stuffs Regulations 1976¹ now require a declaration of total selenium when selenium has been added in the course of the preparation of a compound animal feeding stuff. A method is therefore required for the determination of low levels of selenium in feeding stuffs, the addition declared by compounders being usually in the neighbourhood of 0.1 part per million.

The method initially employed for the determination of low levels of selenium in plant materials and widely used in the early investigations followed the method recommended for selenium in effluents² and was based on the reduction of selenious acid to selenium by ascorbic acid or sulphur dioxide, and assessment of the reddish coloured suspensions obtained against suitably prepared standards.

In 1963 the Society for Analytical Chemistry published methods for the determination of trace elements in feeding stuffs,³ and the process described therein for selenium involved wet ashing followed by treatment with 3,3-diaminobenzidine. The selenadiazole complex thus produced was determined absorptiometrically or fluorimetrically. The method required 50 grams of the feeding stuffs to be employed, large volumes of acids were used in the wet oxidation, and a final distillation from hydrobromic acid was required.

A more sensitive method was described in 1976 by Yasuaki Shimoishi⁴ who found that selenium reacted with 1,2-diamino-4-nitrobenzene to form 5-nitropiazselenol which could be detected by gas chromatography using an electron-capture detector; as little as 0.005 μg of selenium could be detected by this means, and the method was applied to the examination of milk, milk products and albumen.

This method employs a preliminary wet oxidation with nitric acid and after evaporation to a low bulk the remaining oxides of nitrogen are destroyed by treatment with urea. Derivatisation is carried out by the addition of 1,2-diamino-4-nitrobenzene, and after extraction of the 5-nitropiazselenol with toluene the selenium is determined by electron capture. This method was claimed to have the great advantage of working on small quantities of sample and the distillation with hydrobromic acid was unnecessary.

Experimental

The method as described by Shimoishi has been applied in our laboratory to the examination of selenium in compound animal feeding stuffs.

The initial tests were carried out on a pig meal to which no selenium had been added and, with the recorded sensitivity of the method, we employed a standard quantity of approximately one gram in order to detect the required level of 0.1 part per million of selenium, the minimum amount usually declared in some feeding stuffs.

Nitric acid was used as described by Shimoishi for the destruction of the organic matter, but it was found that between 90 and 100 ml of the concentrated acid was required for complete oxidation and, moreover, difficulty was experienced in concentrating the residual acid to a volume of 0.2–0.3 ml as prescribed in the method.

This inability to control the final volume in the evaporation of the nitric acid was undoubtedly the explanation of the very serious losses of selenium (up to as much as 90 per cent.) which were found in some of the early experiments.

The addition of 1 ml of 65 per cent. perchloric acid was then tried and this not only reduced the amount of nitric acid required, but also made the final evaporation much simpler. This latter method of wet ashing was used in the early part of our investigation. The procedure was found to have the required sensitivity, and at levels of 0.1 part per million recoveries of 80 per cent. were obtained.

The whole question of the wet oxidation of organic matter prior to the determination of traces of selenium has recently been studied by the Metallic Impurities in Organic Matter Sub-Committee of the Analytical Division of the Chemical Society.⁵

In view of doubts expressed in some quarters as to the wisdom of employing perchloric acid in wet ashing, attempts were made to obtain an alternative procedure.

We were shown a draft EEC method⁶ for the determination of selenium in feeding stuffs based on a fluorimetric finish in which a preliminary ashing with magnesium nitrate (after nitric acid treatment) was used to obtain a solution of the feeding stuff. The method as prescribed involved digestion with nitric acid overnight, followed by boiling under reflux for 4 to 5 hours, transference to a porcelain dish, the addition of magnesium nitrate solution, evaporation, several periods of controlled temperature drying, followed by ashing at 500°C and final dissolution in hydrochloric acid.

The tests showed that this procedure could be very considerably shortened and a short modification involving treating the 1 g sample with magnesium nitrate solution and nitric acid, evaporation and controlled ashing was found to be satisfactory. Full details are described under the description of the method.

Having devised a modified procedure for the nitric acid-magnesium nitrate ashing method for the destruction of organic matter it was found that a similar method had been described for the determination of traces of selenium in biological material by C. F. Poole *et al.*⁷ that was based on earlier work by Ihnat.⁴ The relatively small quantity of concentrated nitric acid recommended by these

workers was, however, found to be quite inadequate for animal feeding stuffs and substantially greater quantities were required for these materials.

In the initial experiments the column packing was 15 per cent. of SE30 on 60–80 mesh Chromosorb W. With this packing animal feeding stuffs gave a number of peaks and the 5-nitropiazselenol peak was broad and inclined to overlap another peak. A number of alternative column packings of varying polarity were then tried out. When the column packing, however, was changed to 5 per cent. of OV 275 on 60–80 Chromosorb W HP the number of peaks was considerably reduced and the selenium appeared as an isolated sharp peak; the separation and sensitivity were very satisfactory.

Yasuaki Shimoishi showed that 1,2-diamino-4-nitrobenzene only reacts with selenium (IV) and not with selenium (VI); moreover, the nitric acid digestion recommended does not alter the state of oxidation of the selenium present in a mixture. Treatment of the final solution after digestion, with hydrochloric acid will, however, convert Se (VI) to Se (IV) which will then be detected by the method, and a measure of Se (VI) and Se (IV) separately thus becomes possible.

When solutions containing selenium (VI) are heated with hydrochloric acid a reduction to selenium (IV) takes place according to the equation:



This reaction is usually carried out in approximately 7.5N hydrochloric acid, and on a water bath at a temperature not exceeding 100°C. If hydrochloric acid of a greater strength is used or if the temperature rises above a 100°C then selenium is lost as a volatile chloride $\text{SeOCl}_2 \cdot \text{SeO}_2 \cdot 2\text{HCl}$ and the selenium volatilised may have been present originally as either selenium (IV) or selenium (VI).

These reactions have been confirmed and when 7.5 N hydrochloric acid containing selenium is distilled, selenium has been recovered from the distillate.

The final procedure adopted was as follows.

REAGENTS

1. *1,2-Diamino-4-nitrobenzene dihydrochloride solution*, 1 per cent. This compound is sold in the U.K. as 3,4-diamino-nitrobenzene and should be purified as follows. Dissolve 10 g of the technical grade material in M hydrochloric acid, add 2 g of activated charcoal, stir for 5 minutes and filter using a Buchner funnel. To the filtrate add an equal volume of concentrated hydrochloric acid, stir, allow to stand 15 minute and filter on a Buchner. Dry overnight in a vacuum desiccator. Dissolve 1 g of purified material in 100 ml of M hydrochloric acid.
2. *Selenium (IV) stock solution*. Dissolve 0.704 g of selenium (IV) oxide in 500 ml of distilled water—1 ml contains 1 mg of Se.
3. *Toluene*, Analar grade.
4. *Hydrochloric acid*, 7.5 M.
5. *Sodium hydroxide*, M.
6. *Magnesium nitrate solution*, 40 per cent. Dissolve 40 g of magnesium nitrate Analar in water and make up to 100 ml.
7. *Concentrated nitric acid*, Analar.

GAS CHROMATOGRAPHY

Column	2.4 m $\times$ 4 mm i.d. borosilicate glass.
Packing	5 per cent. OV 275 on 60-80 Chromosorb W H.P.
Column temperature	195°C Injection and detector temperature 250°C. On column injection is advisable.
Gas flow	50 ml/min nitrogen.
Detection	Electron capture.

Procedure

The sample submitted for examination should be ground to pass a 1 mm sieve. Since the levels of selenium to be detected are very low and thorough mixing is essential.

Place 1 g of the sample $\pm$ 0.01 g in a 70 ml silica basin, add 5 ml of magnesium nitrate solution, 10 ml of concentrated nitric acid and mix well with a small rod. Evaporate to dryness on a water bath, add a further 20 ml of nitric acid and again mix with a rod. Evaporate to dryness on a water bath and when dry, heat gently on a hot plate until the initial vigorous reaction is completed and then continue the heating over a low argand burner or in a muffle at 500°C. To the white ash, when cold, add 5 ml of water and 7 ml of concentrated hydrochloric acid; immediately cover with a watch glass and heat on a boiling water bath for 20 minutes.

Cool, transfer to a separator, wash in with 7 ml of 7.5 M hydrochloric acid and make up to approximately 25 ml with 7.5 M HCl. Add 2 ml of 3-4 di-amino-nitrobenzene reagent and allow to stand for 2 hours. Extract the selenium complex with 2 ml of toluene; run off the toluene layer and wash with 4 ml M of sodium hydroxide followed by 4 ml of 7.5 M hydrochloric acid. Transfer the toluene layer to a small test tube and shake it with approximately 0.5 g of anhydrous sodium sulphate to remove water. Inject 1-5 μ l into the gas chromatogram and compare with standards prepared by adding known amounts of standard selenium (IV) solution to 25 ml of 7.5 N hydrochloric acid and treating each as described above.

Results

The following results show that a satisfactory recovery of selenium is obtained by this method at the levels to be expected in animal feeding stuffs.

TABLE I
SELENIUM RECOVERY FROM SPIKED SAMPLES OF ANIMAL FEEDS

Sample	Total Se (IV) found, parts per million	Se (IV) recovered, parts per million
Reagent blank	nil	
Feed blank A	0.14	
Feed blank A + 1 p.p.m. Se (IV)	1.15	1.01
Feed blank A + 0.5 p.p.m. Se (IV)	0.64	0.50
Feed blank A + 0.2 p.p.m. Se (IV)	0.35	0.21
Feed blank B	0.09	
Feed blank B + 2 p.p.m. Se (IV)	2.14	2.05
	2.07	1.98
Feed blank B + 0.2 p.p.m. Se (IV)	0.27	0.18
	0.30	0.21
Feed blank B + 0.1 p.p.m. Se (IV)	0.18	0.09
	0.17	0.08

SELENIUM NATURALLY PRESENT IN FEEDING STUFFS

For many years traces of selenium have been regarded as essential for the healthy growth of animals, but in our experience few analysts have appreciated that many compound feeding stuffs contain traces of natural selenium at the levels frequently declared in statutory statements.

Four samples of animal feeding stuffs which were specially prepared for us and to which no selenium had been added were found to contain 0.09, 0.14, 0.16 and 0.17 part per million respectively of selenium.

These results were obtained by the method described in this paper and were also confirmed by fluorimetry using 2,3-diaminonaphthalene as the complexing agent.

Four samples of fishmeal were similarly found to contain 2.5, 3.1, 2.9 and 2.8 parts of selenium per million.

Clearly of all the constituents present in animal feeding stuffs, fishmeal will make the largest contribution to the total selenium present. In the Authors' experience, which covers a large number of samples of feeding stuffs taken under the Act, the selenium found substantially exceeds the often stated figure of 0.1 part per million and this indicates that the manufacturers have ignored the selenium that is naturally present in the feed.

References

1. "The Fertiliser and Feeding Stuff (Amendment) Regulations", S.I. 1976, No. 840, H.M.S.O., London.
2. "Recommended Methods for the Analysis of Trade Effluents", The Association of British Chemical Manufacturers and The Society for Analytical Chemistry, W. Heffer & Sons Ltd, 1958.
3. "Determination of Trace Elements with Special Reference to Fertilisers and Feeding Stuff". The Society for Analytical Chemistry, W. Heffer & Sons Ltd, 1963.
4. Shimoishi, Y., *Analyst*, 1976, **101**, 29.
5. Analytical Methods Committee, *Analyst*, 1979, **104**, 778.
6. Draft EEC Method for the Determination of Selenium in Animal Feeding Stuff.
7. Poole, C. F., Evans, N. J., and Wibberly, D. G., *J. Chromatogr.*, 1977, **136**, 73.
8. Ihnat, M., *J. Ass. Off. Anal. Chem.*, 1974, **57**, 368.

