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Hydride generation atomic absorption spectrometric (HGAAS) determination of selenium in term and preterm infant formulae available in the United Kingdom

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A simple and sensitive hydride generation atomic absorption spectrometry method is described for the determination of total selenium in infant formulae. Decomposition of the composite sample matrix involved overnight digestion with a nitric–perchloric acid mixture (10:2 ml) then heating at 150°C (30 min) and 220°C (60 min). No apparent matrix interferences were encountered. The selenium levels in infant formulae, reported for the first time, ranged from 0.034 to 0.093 μg/g (dry wt) for bovine casein and whey-based term powdered infant products, 0.023–0.093 μg/g (dry wt) for preterm powdered formulae and 0.027–0.049 μg/g (wet wt) for hospital administered low birth weight ready-to-feed formulae. These values are dependent on geographic origin of bovine milk and exhibit variation between batches. Routine QC analysis and fortification by manufacturers is recommended.

INTRODUCTION

Within the past two decades, human selenium (Se) deficiency has been identified and much evidence exists to suggest that Se has a developmental role similar to any other essential trace element known to man. Low selenium intake has been associated with two diseases in children: Keshan disease, a cardiomyopathy that primarily affects children aged 2–10 years, and Kashin–Beck disease, an osteoarthritis in preadolescents or adolescents (Levander, 1989).

Neonates, particularly those of low birth weight, may be at greatest risk of selenium deficiency. These infants have lower hepatic stores and plasma concentrations than term infants and are vulnerable to haemolytic anaemia, heart disease and cancer in later life (Reifen & Zlotkin, 1993).

Previous data suggest that term infants which are fed formula (2–4 μg Se intake/day) as opposed to breast milk (5–13 μg Se intake/day) as their sole source of nutrition may be at risk of selenium deficiency (Lombeck *et al.*, 1978; Smith *et al.*, 1982). In the United States, infant formula is fortified with selenium in the form of sodium selenite (Goedhart & Bindels, 1994). However to date, UK-based manufacturers are not required to supplement or monitor the selenium content of infant formulae; levels present being those naturally occurring in milk. However, the selenium content of milk is low (0.004–0.02 μg/g) (Thorn *et al.*, 1978; Koops *et al.*, 1989) and cannot provide enough selenium compared to the recommended reference nutrient intake (RNI) of 10 μg/day (Department of Health, 1991). Owing to lack of sufficient data, precise amounts recommended for selenium intakes for infants in Great Britain (estimated average requirements) have not yet been defined.

No British data on infant formulae has been reported with respect to selenium and very little information is generally available on the selenium content of infant formulae from other parts of the world, or its method of analysis (Zabel *et al.*, 1978; Amin *et al.*, 1980; Lewis *et al.*, 1985). A recent review of selenium determination in milk and infant formulae (Foster & Sumar, 1995) describes the most commonly used current methods of analysis. In this review concern is expressed over the carcinogenic nature of 2,3-diaminonapthalene (DAN) which has led to a move away from this classical fluorometric technique in search of alternative procedures. Of

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these, hydride generation atomic absorption spectrometry (HGAAS) offers excellent promise in terms of sensitivity (0.02–2 ng), minimal matrix interferences and relatively inexpensive equipment requirements (Tingii *et al.*, 1992; Verber *et al.*, 1994). Matrix interferences from transition metals, particularly copper, can cause significant negative errors in animal tissue, although this is unlikely in dairy-based foods (Hershey & Oostdyk, 1988). Similarly, no interferences are observed with sodium, potassium, calcium magnesium and phosphorous which are abundant in the milk matrix (Noda *et al.*, 1981). The most common of the HGAAS methods involves reduction of selenium by sodium borohydride followed by introduction of the hydride by means of a carrier gas (nitrogen) directly into an air-entrained nitrogen–hydrogen flame (Jackson & Qiao, 1992).

Quantitative analysis of selenium in aqueous solution requires complete destruction of the composite organic matrix. Typically wet oxidation procedures use a variety of oxidant mixtures, i.e. nitric acid–perchloric acid (HNO_3–$HClO_4$), nitric acid–hydrogen peroxide (HNO_3–H_2O_2), nitric acid–sulphuric acid (HNO_3–H_2SO_4) and nitric acid–perchloric acid–sulphuric acid (HNO_3–$HClO_4$–H_2SO_4). The choice of acid assumes complete conversion of native forms of selenium in the matrix to selenite–selenate; reconversion of native forms of selenium to selenite and the prevention of significant loss of selenium by volatilization (Janghorbani *et al.*, 1982). For milk analysis, HNO_3–$HClO_4$ forms the preferred acid mixture as it gives optimum recoveries of selenium particularly when nitric acid is the predominant acid (Ting *et al.*, 1982). Perchloric acid facilitates the oxidation of resistant fatty material and organoselenium compounds (Subramanian & Meranger, 1982). Losses and interference can be minimized by not taking the digestion to complete dryness. Hazards associated with this mixture are often minimized by using tertiary mixtures (HNO_3–$HClO_4$–H_2SO_4). Whilst suitable for digesting a wider range of mixtures, the addition of sulphuric acid enhances the risk of charring and loss of selenium through volatilization (Haddad & Smythe, 1974). Hydrochloric acid is the usual reducing agent for the conversion of Se(VI) to Se(IV) at elevated temperature for 30–60 min to minimize losses and low recovery (Lalonde *et al.*, 1982).

The purpose of this study is to develop a simple hydride generation spectrometric method for infant formulae analysis to replace the classical fluorimetric technique. This method will be applied to determine the total selenium in formula with a view to assessing the variation in selenium content of term and preterm infant formulae currently available in the UK.

MATERIALS AND METHODS

Samples

Seven leading brands of infant formulae were purchased at retail outlets across South London or directly from the manufacturer during the spring of 1995. Several quantities of each type, bearing different sell by dates and batch numbers were also acquired. Formulae samples were stored in the absence of light at ambient temperature. On the day of analysis, the tin seal was broken and a representative sample obtained by mixing and quartering. In total, 102 samples were analyzed.

Sample digestion

Representative milk samples (0.25 g powder; 1.0 ml liquid) were placed in Pyrex glass digestion tubes, specially designed, with ground glass B19 joints and stoppers, long neck with dimensions of 24 × 235 mm and a nominal capacity of 56 ml which had been previously soaked overnight in 10% v/v nitric acid (AnalaR, BDH-Merck Ltd, Poole, Dorset, UK), rinsed in double distilled, deionized water (15–18 M specific resistivity, Elgastat, UK) and acetone (AnalaR, BDH-Merck Ltd) then dried with compressed air. The water content of liquid samples was reduced by gently heating the tubes in a waterbath at 100°C.

Samples of 0.25 g (dry wt) non-fat milk powder National Institute of Standard and Technology standard reference material 1549 (Laboratory of the Government Chemist, Teddington, UK) were also treated similarly thoughout the entire digestion process. The following volume ratios of acids (Aristar, BDH-Merck Ltd) were added and stoppered tubes left in a class A fume cupboard at ambient temperature for digestion overnight: (i) 5.0 or 10.0 ml nitric acid (S.G. 1.43 g/ml)–perchloric acid (S.G. 1.70 g/ml) mixture (4:1); or (ii) 10 ml nitric acid. The following day, the tubes were placed in an ultrasonic bath for 2 min, a few acid washed, rinsed antibumping granules (fused silica, BDH-Merck) added to each and 2 ml perchloric acid added to (ii). The tube contents were heated to 150°C (without stoppers) in a Kjeldatherm digestion block (Gerhardt UK Ltd, Cheshire, UK) for approximately 30 min until the evolution of brown fumes of NO_2 had ceased, then gradually increased to 220°C for 45–60 min after the appearance of dense white fumes of perchloric acid.

Care was taken to ensure that the solution was not heated to complete dryness because of the explosive nature of metal perchlorates (Analytical Methods Committee, 1979). Foaming was controlled by passing a jet of compressed air over the mouth of the tubes. A few drops of nitric acid were administered periodically to prevent charring. The digestion was completed when approximately 0.5 ml of colourless solution remained. On cooling, the tubes were made up to 5 ml volume with deionized water, 5.0 ml hydrochloric acid (S.G. 1.18 g/ml, AnalaR, BDH-Merck) added and heated at 100°C for 30 min to reduce Se(VI) to Se(IV).

Hydride generation atomic absorption spectrometry

After the sample digest had cooled, it was transferred into a calibrated tube (25 ml) and diluted to 20 ml with deionized water, placed in the hydride generation

Table 1. Instrumental conditions for atomic absorption measurements and hydride generation

Atomic absorption measurements	
Radiation source:	Selenium hollow-cathode lamp, 7 mA
Wavelength:	196.0 nm
Spectral band pass:	1.0 nm
Measurement mode:	Integrated peak area 120 s
Flame:	Air–acetylene, 10 psi, flow rate 3 litres/min
Mains supply:	240 V
Hydride generation	
Tube temperature:	approx. 900°C
Carrier gas:	Nitrogen at 10 psi
Reaction time:	20 s
Reaction volume:	20 ml

reaction chamber (GBC model HG 900 manual vapour generation system, GBC Scientific Equipment Pty Ltd, Melbourne, Australia) and 5 ml 2.5% w/v sodium borohydride (98% crystalline, Sigma Chemical Company, Dorset, UK), dissolved in 0.1% w/v sodium hydroxide (BDH-Merck, UK), was injected into the chamber. This solution was freshly prepared for every analysis as it is only stable for approximately 2 h. When bubbling occurred, indicating hydrogen evolution and breakdown of reducing agent strength, the solution was discarded. All reagents were handled with plastic utensils to prevent metal contamination. Atomic absorption measurements were performed on a GBC model 502 atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Australia). Instrumental conditions are given in Table 1. The peak area signal was recorded on a strip chart integrator (Spectraphysics, 4270). An external calibration curve was prepared using 1000 ppm Se(IV) selenium standard in 0.5 M HNO_3 ('Spectrosol', BDH-Merck Ltd) over the range 0–0.01 μg/ml from a 1 ppm working stock standard solution using 2.8 M hydrochloric acid (Aristar, BDH-Merck Ltd) as a diluent. Peak area response was plotted against the Se concentrations.

RESULTS AND DISCUSSION

Sample digestion

The effects of varying acid digestion mixtures and heating temperatures are shown in Table 2. Lower heating temperatures resulted in a strong colour retention (yellow–green) at the end of the digestion process, indicating some organic components remained owing to incomplete digestion. In addition, the presence of fat suggests the volume of acid used was insufficient to digest the matrix completely. This was confirmed when the acid volume was doubled as all traces of lipid were absent in the final digest. The addition of antibumping granules reduced charring from localized heating in conjunction with the addition of small volumes (between one and five drops) of nitric acid throughout the heating process. Excessive foaming was a prominant feature of preliminary digest experiments. This was probably owing to the high protein content of infant formulae, as caseins are strongly surfactant in nature. Ultrasonic treatment of the samples reduced this effect by disrupting the molecular structure of these molecules. In addition samples were allowed to stand overnight in nitric acid prior to heating promoting acid hydrolysis. Any further foaming was controlled by passing a stream of compressed air over the surface of the boiling liquid. No perceivable difference was observed between samples left to digest in a nitric–perchloric acid mixture and those left in nitric acid only. For safety reasons nitric acid was added first to destroy any easily oxidizable components, minimizing any risk of explosion (Bock, 1979).

Julshamn *et al.* (1982) reported that high nitric and perchloric acid concentrations can suppress the absorption signals, resulting in a significant reduction in sensitivity and reproducibility. A final digest of > 1 ml was found to suppress the absorption signal resulting from the presence of nitrate which interfered with the formation of the hydride. For this reason the final digest volume was reduced to 0.5 ml which ensured the digest acids were kept below 4% in the final volume sample dilution and minimized acid interferences.

Hydride generation atomic absorption spectrometry

The reaction time required for the atomic absorption of selenium after the addition of sodium borohydride was established by observing the change in absorbance signal over the period of 0–180 s as the reaction progressed. After the initial reaction on injection, the signal increased gradually up to a maximum value at 120 s. This was probably owing to the slow removal of the last traces of hydride from the solution. After this point

Table 2. Observed effects of varying acid mixtures and temperature on sample decomposition

Concentrated acid proportions	Heating conditions	Observed effect
(i) 5 ml HNO_3–$HClO_4$ (4:1)	150°C for 30 min	strong colour retention
	(a) 190°C for 100 min	fat visible on cooling
	or	
	(b) 220°C for 60 min	yellow colouration foaming, charring
(ii) 10 ml HNO_3–$HClO_4$ (4:1)	150°C for 30 min	foaming, slight charring
	220°C for 45-60 min	clear solution

Table 3. Selenium concentrations (μg/g dry wt) in UK available infant formulae

Infant feed	Area of manufacture	n^a	SE[b]	V^c	Mean ± SD[d]
Term formulae					
Cow & Gate Plus (casein dominant)	Ireland	18	0.006	0.0006	0.053 ± 0.02
Cow & Gate Premium (whey dominant)	Ireland	18	0.007	0.0009	0.061 ± 0.03
Preterm formulae					
Cow & Gate Nutriprem 2	Ireland	18	0.01	0.0017	0.033 ± 0.03
Farleys Premcare	Ireland	18	0.008	0.0011	0.045 ± 0.02
Milupa Prematil	Denmark	18	0.007	0.0008	0.083 ± 0.01
Ready-to-feed formulae[e]					
Cow & Gate Nutriprem	Ireland	6	0.004	0.0001	0.049 ± 0.004[f]
SMA Low Birth Weight	England	6	0.002	0.0001	0.027 ± 0.01[f]

[a] n = number of samples analyzed.
[b] SE = standard error (μg/g).
[c] V = variance (μg/g).
[d] Mean with standard deviation (SD).
[e] Hospital use only.
[f] Concentration expressed on a wet weight basis.

there was a slow reduction in signal, but the loss was relatively small. This observation is in agreement with Brooks *et al.* (1983).

The efficiency of hydride production was found to be dependant on the hydrochloric acid concentration. As no interferences were apparent, concentrations between 0.5 and 4 M HCl were investigated. For this system, a concentration of approximately 2.8 M produced an optimim level of efficiency.

During the initial analyses, poor reproducibility was achieved and difficulties encountered with instrument stabilization between readings. A dramatic improvement was effected by washing the reaction chamber and quartz tube with 10% v/v nitric acid, deionized water, acetone and drying with compressed air between samples. It is apparent that adequate washing is a vital prerequisite for reproducible work, since selenium has a tendency to form a cumulative deposit on the exposed surface of the reaction chamber as the hydride is formed.

The calibration graph peak area response was directly proportional to the selenium (IV) concentration over the described range (correlation coefficient = 0.997). the coefficient of variation was 3.54% ($n = 7$).

Accuracy and detection limit

The accuracy of the method was assessed by analysing non-fat milk powder standard reference material (National Institute of Standard and Technology SRM 1549, Teddington, UK). The results obtained were 0.11 ± 0.008 μg/g (coefficient of variation of 7.12%; $n = 7$) which is in excellent agreement with the certified value (0.11 ± 0.01 μg/g). The detection limit for HGAAS was 0.15 ng/ml (coefficient of variation of 3.54%; $n = 7$).

Background analytical levels of selenium were assessed by running blank acid digestions. The levels detected were insignificant.

Selenium content of infant formulae available in the United Kingdom

The levels and batch variation of selenium found in seven leading brands of infant formulae purchased in 1995 are shown in Table 3 and Fig. 1, respectively. The results, blank corrected, are expressed in μg/g (dry weight) powder and μg/g (wet weight) for the ready-to-feed formulae.

Concentrations of selenium in the term formulae ranged from 0.034 to 0.093 μg/g, mean 0.057 μg/g. The amount of Se in the whey dominant type (0.061 μg/g)

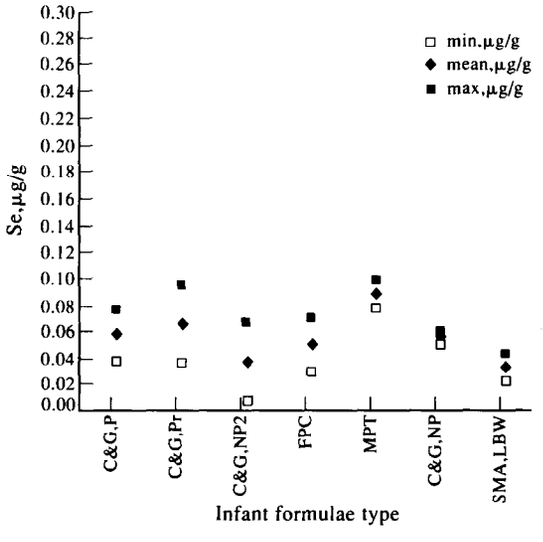

Fig. 1. Se levels between batches of commercially available UK infant formulae.

was slightly higher than that of the casein dominant type (0.053 μg/g). This result is surprising as Van Dael *et al.* (1991) reported that 55% of the total selenium in Belgium bovine milk was associated with the casein fraction, as determined by HGAAS. However, an earlier report by Deschuytere *et al.* (1987) found whey protein to be the predominant source of selenium. For both formulae the variation in selenium content between batches was wide, and of a similar range. The Se content of the casein dominant product ranged from 0.034 to 0.073 μg/g and from 0.037 to 0.095 μg/g in the whey dominant product, respectively.

The levels of selenium shown in Table 3 for term formulae are comparable to those reported in other countries. In Germany, reported levels (dry weight) for milk-based formulae analysed using fluorimetry and neutron activation analysis were 0.009–0.098 and 0.018–0.171 μg/g, respectively (Zabel *et al.*, 1978; Lombeck *et al.*, 1978). A later study in the United States by Smith *et al.* (1982) used gas chromatography with electron capture detection to analyse a wide range of commercially available infant formulae. The reported levels ranged from 0.022 to 0.034 μg/g (dry weight), based on 76.24% moisture content. This variation in selenium levels for formulae from different countries relates to the geographic origin of the raw material. For bovine milk, the selenium content is dependent on the amount and availability of the element to plants for cattle grazing, the former being directly influenced by the aqueous solubility of the element (Combs, 1988).

Concentrations of selenium in preterm powdered formulae ranged from 0.023 to 0.093 μg/g, mean 0.054 μg/g. For the hospital administered ready-to-feed formulae, concentrations ranged from 0.027 to 0.049 μg/g, mean 0.038 μg/g. These values are in agreement with Amin *et al.* (1980), who determined an average selenium concentration of 0.06 μg/g for US preterm formulae. It is important to note that the average values obtained for Milupa Prematil (0.083 μg/g) are generally higher than the other formulae analyzed (0.033–0.045 μg/g). This is probably owing to the fact that the bovine milk originates from Denmark, rather than the United Kingdom.

Bearing in mind the vulnerability of preterm and low birth weight infants to selenium deficiency, the lack of observed uniformity between batches (Fig. 1) is significant, particularly if one considers that such feeds provide the only source of this element for formulae-fed infants.

It is evident that the geographic origin of bovine milk and its handling by different manufacturers influences the Se content of infant formulae. Currently UK manufacturers are not required to monitor the selenium content of infant formulae. However, our data suggest that variation in selenium levels between batches does exist. Owing to the inadequate hepatic Se stores of preterm infants, and thus increased risk of deficiency, it is recommended that infant formulae, particularly preterm and low birth weight feeds, should be analysed for selenium. The proposed method provides a simple and sensitive technique for the determinaton of selenium in infant formulae.

In addition, any variation between batches could be remedied by fortification with sodium selenite, as is current practice in the United States, to increase concentrations similar to those of mature human milk, i.e. 0.1–0.23 μg/g (Department of Health, 1991; Lombeck *et al.*, 1978). In Europe, infant formulae supplementation is currently under consideration, although the addition of selenium has to await the formalization of the latest 1993 EC Directive (Goedhart & Bindels, 1994).

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REFERENCES

Amin, S., Chen, S. Y., Collipp, P. J., Castro-Magana, M., Maddaiah, V. T. & Klein, S. W. (1980). Selenium in premature infants. *Nutr. Metab.*, **24**, 331–40.

Analytical Methods Committee (1979). Report prepared by the Metallic Impurities in Organic Matter Sub-committee. Determination of small volumes of selenium in organic matter. *Analyst*, **104**, 778–87.

Bock, R. (1979). *A Handbook of Decomposition Methods in Analytical Chemistry, International Textbook*. Blackie Group, Glasgow.

Brooks, R. R., Willis, J. A. & Liddle, J. R. (1983). Optimum conditions for hydride generation of selenium and its determination by atomic absorption spectrometry. *J. Assoc. Off. Analyt. Chem.*, **66**, 130–4.

Combs, G. F. (1988). Selenium in foods. *Adv. Food Res.*, **32**, 85–113.

Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. Report on Health and Social Subjects 41. HMSO, London.

Deschuytere, A., Vermwylon, K. & Deelstra, H. (1987). Vitamin E and selenium concentrations in milk and milk powder. *Z. Lebensm. Unters. Forsch.*, **184**, 385–7.

Foster, L. H. & Sumar, S. (1995). Methods of analysis used for the determination of selenium in milk and infant formulae: a review. *Food Chem.*, **53**, 453–66.

Goedhart, A. C. & Bindels, J. G. (1994). The composition of human milk as a model for the design of infant formulas: recent findings and possible applications. *Nutr. Res. Rev.*, **7**, 1–23.

Haddad, P. R. & Smythe, L. E. (1974). A critical evaluation of fluorimetric methods for determination of selenium in plant materials with 2,3-diaminonapthalene. *Talanta*, **21**, 859–65.

Hershey, J. W. & Oostdyk, T. S. (1988). Determination of arsenic and selenium in environmental and agriculteral samples by hydride generation atomic absorption spectrometry. *J. Assoc. Off. Analyt. Chem.*, **71**, 1090–3.

Jackson, K. W. & Qiao, H. (1992). Atomic absorption, atomic emission and flame emission spectrometry. *Analyt. Chem.*, **64**, 50R–66R.

Janghorbani, M., Ting, B. T. G., Nahapeltian, A. & Young, V. R. (1982). Conversion of urinary selenium to selenium (IV) by wet oxidation. *Analyt. Chem.*, **54**, 1188–90.

Julshamn, K., Ringdal, O., Slinning, K. & Braeklan, O. R. (1982). Optimization of the determination of selenium in marine samples by atomic absorption system with a hydride generation atomic absorption system. *Spectrochim. Acta*, **B37**, 473–82.

Koops, J., Klomp, H. & Westerbeek, D. (1989). Determination of selenium in milk by spectrofluorimetry and by Zeeman-corrected, stabilised temperature platform furnace atomic atomic absorption spectroscopy. Comparison of results. *Neth Milk Dairy J.*, **43**, 185–98.

Lalonde, L., Jean, Y., Roberts, K. D., Chapdelaine, A. & Bleau, G. (1982). Fluorimetry of selenium in serum or urine. *Clin. Chem.*, **28**, 72–4.

Levander, O. A. (1989). Upper limit of selenium in infant formulas. *J. Nutr.*, **119**, 1873–89.

Lewis, S. A., Patterson, K. Y., Hardison, N. W., Watson, J. & Veillon, C. (1985). The analysis of infant formulae for total selenium by isotope dilution mass spectrometry. *Proc. of the AOAC Conference on Production, Regulation, and Analysis of Infant Formula.* Association of Official Analytical Chemists, Virginia Beach, Virginia, pp. 227–32.

Lombeck, I., Kasperek, K., Bonnermann, B., Feinendegen, L. E. & Bremer, H. J. (1978). Selenium content of human milk, cow's milk and cow's milk infant formulas. *Eur. J. Pediatr.*, **129**, 139–45.

Noda, K., Kenjo, N. & Takahashi, T. (1981). Determination of selenium in milk powder by atomic absorption spectrophotometry. *Nippon Shokuhin Kogyo Gakkaishi.*, **23**, 260–3.

Reifen, R. M. & Zlotkin, S. (1993). In *Nutritional Needs of the Preterm Infant*, eds R. Vavy, S. Zlotkin, R. C. Tsang & A. Lucas. Williams and Wilkins Inc., Baltimore, Maryland.

Smith, A. M., Picciano, M. F. & Milner, J. A. (1982). Selenium intakes and status of human milk and formula fed infants. *Am. J. Clin. Nutr.*, **35**, 521–6.

Subramanian, K. S. & Meranger, J. C. (1982). Rapid hydride evolution—electrochemical atomisation absorption spectrophotometric method for determining arsenic and selenium in human kidney and liver. *Analyst*, **107**, 157–62.

Thorn, J., Robertson, J. & Buss, D. H. (1978). Trace nutrients. Selenium in British food. *Br. J. Nutr.*, **39**, 391–6.

Ting, B. T. G., Nahapeltian, A., Young, V. R. & Janghorbani, M. (1982). Conversion of tissue selenium to selenium (IV) by wet oxidation. *Analyst*, **107**, 1495–8.

Tingii, V., Reilly, C. & Patterson, C. M. (1992). Determination of selenium in foodstuffs using spectrofluorimetry and hydride generation atomic absorption spectrometry. *J. Food Composit. Anal.*, **5**, 269–80.

Van Dael, P., Vlaemyrick, G., Van Renterghern, R. & Deelstra, H. (1991). Selenium content of cow's milk and its distribution in protein fractions. *Z. Lebensm, Unters. Forsch.*, **192**, 422–6.

Verber, M., Cujes, K. & Gormiscek, S. (1994). Determination of selenium and arsenic in mineral waters with hydride generation atomic absorption spectrometry. *J. Analyt. Atom. Spectrom.*, **9**, 285–90.

Zabel, N. L., Harland, J., Gormican, A. T. & Ganther, H. E. (1978). Selenium content of commercial formula diets. *Am. J. Clin. Nutr.*, **31**, 850–8.