Determination of Selenium in Biological Media

1. SCOPE

The method is suitable for the determination of total selenium in biological tissue at levels of the order of 0.01-0.03mg/kg.

2. PRINCIPLE

The sample is digested under strongly oxidising conditions with nitric and perchloric acids, a fluorescent selenium complex (naphtho[2,3-d]-2-selena-1,3-diazole) formed with 2,3-diaminonaphthalene, extracted into cyclohexane, back-extracted into 0.1 M hydrochloric acid and compared with standard selenium solutions spectrofluorimetrically.

3. REAGENTS

Perchloric acid, 72%; Hydrochloric acid, concentrated; Nitric acid, concentrated–redistilled; Ammonium hydroxide solution, 7 M; Cyclohexane; dekalin (decahydronaphthalene); Light petroleum–boiling range 100-120°C; EDTA solution–0.04 M diammonium salt prepared from EDTA and ammonium hydroxide solution; 2,3-Diaminonaphthalene (DAN)–recrystallised from water in diffuse daylight with a little sodium sulfite and activated charcoal.

DAN solution–0.1% in 0.1 M hydrochloric acid, prepared immediately before use in yellow light. Dissolve 0.05g 2,3-diaminonaphthalene in 50ml 0.1 N hydrochloric acid, heat at 50°C for 20 minutes, cool to 20°C: extract twice with 10ml dekalin, centrifuge (10.1).

Cresol Red Solution–0.02% w/v in water.

Standard Se solution–Dissolve an accurately weighed amount of selenium (Se) in a minimum amount of concentrated nitric acid, evaporate off the excess nitric acid in the presence of a little perchloric acid and add a trace of hydrochloric acid to give a solution containing 0.1 N acid. Dilute to 0.1 microgram Se per ml with 0.1 N hydrochloric or perchloric acid.

4. APPARATUS

Filter fluorimeter; digestion tubes, 2.5 x 20cm, fitted with 15cm air condensers; evaporator–manifold connected to centrifugal pump, connected to digestion tubes immersed in a water bath; separatory funnels–100ml; centrifuge.

5. PROCEDURE

Digest ca. 1.0 gram of sample with 5ml nitric acid, adding more nitric acid then 2ml perchloric acid when the initial vigorous reaction ceases. Reflux, fume and boil strongly for 15 minutes before cooling. Add 2ml water, evaporate, add 2ml hydrochloric acid, heat in boiling water bath for 5 minutes, rinse internally with 4ml water, cool.

To each tube (including blanks and standards) add 2ml EDTA solution, one drop of cresol red solution and adjust to pH 1.0 with 7 M ammonium hydroxide solution (or dilute perchloric acid solution). Dilute to 50ml with 0.1 M hydrochloric acid, add 5ml DAN solution, stopper, shake and leave in a water bath at 50°C in the dark for 20 minutes. Cool in a water bath at room temperature in diffuse daylight, run into a 100ml separatory funnel containing 10ml cyclohexane, shake for one minute, discard the aqueous phase. Extract the cyclohexane phase twice with 25ml 0.1 M hydrochloric acid, separate and centrifuge (2,000rpm for 2 minutes) in polypropylene centrifuge tubes, measure fluorescence (6.2).

Notes: Oxygen flask combustion may also be used (6.2).

6. REFERENCES

6.1 Parker, C. A. and Harvey, L. G., *Analyst*, 1962, **87**, 558.

6.2 Watkinson, J. H., *Anal. Chem.*, 1966, **38**, 92-97.