Determination of selenium after derivatization with 2,3-diaminonaphthalene in water and serum

A-26.2

Key words

Instrumental HPTLC - quantitative analysis - prechromatographic derivatization - densitometry (fluorescence) - selenium - drinking water - toxicology - diagnostics

Scope

By wet treatment with chemicals and reduction, selenium is conversed to selenite and derivatized to 2,1,3-naphthoselenodiazol. It is then extracted by Extrelut-columns. The eluate is chromatographed on silica with chloroform. To increase fluorescence intensity the plate is dipped in a solution of Triton X-100 and then evaluated quantitatively by fluorescence measurement.

Literature

[1] W. Funk, V. Dammann, Th. Coutourier, J. Schiller, L. Völker: J. High Resol. Chromatog. **9,** 222-235 (1986).

[2] W. Funk, V. Dammann, H. Vogt: in "Instrumentelle HPTLC", A. Hüthig Verlag, Heidelberg, 321-336 (1982).

[3] J.Th. Schiller, Th. Coutourier: diploma thesis, Faculty of Occupational Health, Polytechnic of Giessen, 1982 and 1984.

Advantages of using HPTLC for this analytical task

- Limit of detection in the femtogram range (250 fg)
- Simple procedure
- Cost effectiveness

Chemicals

Water (bidist.)
HNO_3 (65%)
H_2O_2 (30%)
HCl (25%)
EDTA solution (3.7%)
NaF (1.4%)
Formic acid (50%)
NH_3 (25%)
Cyclohexane
Butylhydroxytoluene
Chloroform
Methanol
Ethanol (abs.)
Triton X-100
2,3-diaminonaphthalene (DAN , recrystallized)*
Extrelut column (Merck No. 11737)

Standard: Titrisol (Merck No 9915)

Sample preparation

1. Conversion of sample and standard by wet chemical treatment

- An amount of 0.5 to 5.0 mL sample or 2.5 mL standard solution is evaporated to dryness at 100°C.
- After cooling to 20°C, the residue is dissolved in 5 mL HNO_3 and heated at 110°C for 60 min.
- Three 2 mL portions of H_2O_2 are added at 15 min intervals, and the reaction mixture is left at 110°C.
- The clear solution is then evaporated to dryness at 120°C and the colourless precipitate is taken up with 2 mL H_2O_2 and again evaporated to dryness.
- The residue is dissolved in 20 mL HCl and heated to 110°C for 45 min to reduce the selenate to selenite.

☞ *If this sample is left to stand for prolonged periods (30-60 min), chlorine is evolved and can oxidise the selenite back to selenate. It is advisable to remove the chlorine by purging with nitrogen.*

2. Derivatization of sample and standard

- To mask interfering ions, 1 mL aqueous EDTA solution, 0.5 mL aqueous NaF solution, and 2 mL formic acid are added to the solution.
- The solution is adjusted to pH 1.8 with NH_3 (or HCl), and 2 mL DAN solution is added.
- The mixture is then heated to max. 40°C for a few minutes in the dark, adjusted to pH 6 with NH_3, transferred to a 250 mL (sample) or 500 mL (standard) measuring flask, and filled up to the mark with water (pH = 6).

3. Extraction of sample and standard

- A 20 mL portion of the derivatized sample/standard is transferred to an Extrelut column and after about 12 min eluted with 40 mL cyclohexane (containing 0.01% butylhydroxytoluene for stabilization).
- The eluate (about 25 mL) is collected in a brown or otherwise light-protected tapered-neck flask and evaporated to dryness in a rotary evaporator at max. 35°C.

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* Recrystallization of DAN and preparation of the DAN solution:

1 g DAN is dissolved in 40 mL ethanol (abs.) at 80°C, the solution is passed through a porcelain filter, and the DAN is allowed to crystalline out. The mixture is then filtered through a glass frit (No. 4), the precipitate is washed with 50% ethanol (cold), then dried (brown crystals with a silky sheen).

0.5 g recrystallized DAN is dissolved in 100 mL 0.1 N HCl by heating, extracted three times in a separating funnel with 10 mL cyclohexane, then filtered through a glass frit (No. 4). This solution must be made up fresh each time before use.

- The residue is dissolved in 1 mL chloroform, and quantitatively transferred to a curved rim beaker (this operation is repeated three times).
- The combined chloroform phases are evaporated to dryness at room temperature under nitrogen, and the residue is dissolved in 500 µL chloroform.

Standard solution

Make up one ampoule Titrisol (Merck No. 9915, SeO_2) to 1 L with bidistilled water. Dissolve 50 µL of this mixture in 5 mL water, and proceed as outlined in sample preparation. Make up quantitatively to 1000 mL (1 µL = 5 pg Se) .

Layer

HPTLC plates silica gel Merck 60 F_{254}, 20x10 cm

To improve the precision of the method, the HPTLC plates are prewashed *three times* in succession by blank chromatography with a mixture of methanol - chloroform 1:1, then dried for 20 min at 110°C.

Sample application

With CAMAG Linomat as 7 mm bands, distance between tracks 3 mm, distance from left edge 20 mm, distance from lower edge 8 mm = 16 application per plate, delivery rate 4 s/µL.

Application pattern:

S1 U1 S2 U2 S3 U3 S4 U4 S1 U1 S2 U2 S3 U3 S4 U4

S1 - S4 = 1, 3, 6, 10 µL standard solution , U1 - U4 = 1 µL unknown each.

Chromatography

In CAMAG Horizontal Developing chamber with chloroform (0.01% butylhydroxytoluene as antioxidant). Migration distance 50 mm, running time 9 min, R_F = 0.5.

Derivatization (Intensification of fluorescence)

By dipping the still wet plate three times for 2 s each in a solution of Triton X-1OO - chloroform 1:4 with CAMAG Immersion Device. Between each dip the plate is left for 15 min at room temperature *in the dark* to allow the solvent to evaporate.

For quantitative evaluation the solvent has to be evaporated completely after the final dip (absence of odour). The fluorescence intensity is increased by a factor of 90, and stabilized for about 9 hours.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by fluorescence at 366/>560 nm.

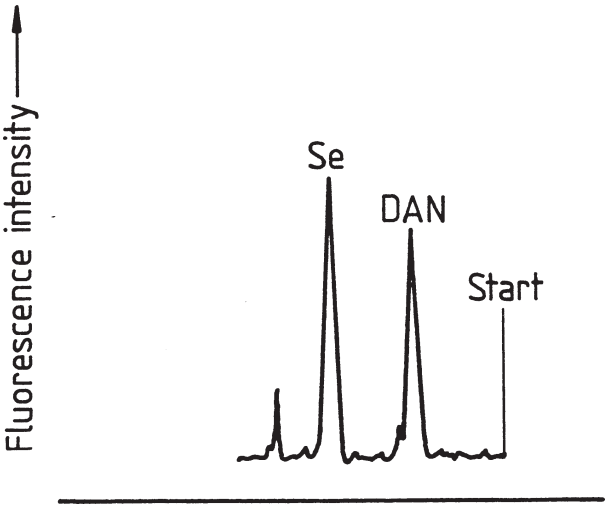

Fig. 1

Densitogram of the fluorescence measurement at 366/>560 nm. Se = 10 pg 2,1,3-naphthoselene-diazol, DAN = 2,3-diaminonaphthalene.

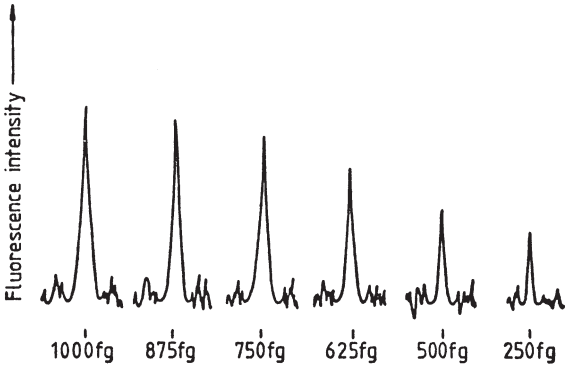

Fig. 2

The limit of determination for this method is about 1 pg Se/spot and the limit of detection is about 250 fg Se/spot.

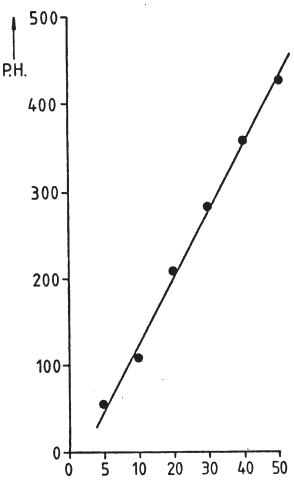

Fig. 3

The linear regression of 2,1,3-naphthoselendiazol shows that the decomposition and extraction procedure does not lead to constant-systematic errors.

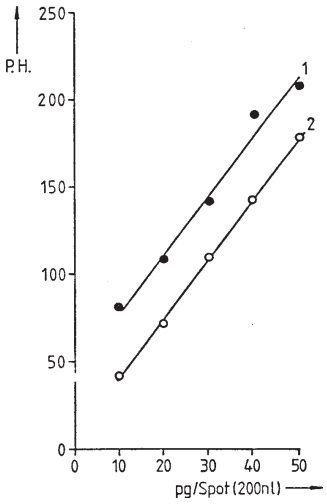

Fig. 4

The standard addition curve (1) is compared with the calibration curve (2) of analogously treated selenocystine standards. The recovery rate is 100.2%. A statistical check of this rate shows that this method is free from any systematic errors.