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DETERMINATION OF SELENIUM IN DRUGS BY OXYGEN FLASK COMBUSTION AND ION CHROMATOGRAPHY

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SUMMARY

A method is described for the determination of trace selenium (impurities in medicinal organic compounds) by ion chromatography (IC) after oxygen flask combustion. All selenium compounds formed by oxygen flask combustion are converted to selenate ion by heating with nitric acid and potassium permanganate. The selenate ion is then determined by IC, using a simple recycle system to eliminate interfering ions. The detection limit of selenium is 0.4 nmol in 50 mg of sample. The recoveries of selenium added to seven drugs are *ca.* 95–103% with relative standard deviations of 1–6%.

INTRODUCTION

A sensitive, accurate and rapid method for the determination of trace selenium is required because of its environmental and physiological importance. In the pharmaceutical sciences, the analysis of drugs for selenium is provided in the JP XI¹ and USP XXI².

The determination of selenium in organic matter generally involves decomposition of the sample by wet digestion with an oxygen-containing acid^{3,4} (*e.g.*, nitric acid and/or perchloric acid) and determination of selenite ion [Se(IV)] by fluorimetry with 2,3-diaminonaphthalene^{3,4} or hydride generation atomic absorption spectrometry³ (HG-AAS).

Recently, we reported a more rapid and accurate method for the determination of selenium in bulk prednisolone⁵. The method is based on oxygen flask combustion of samples, oxidation of the resulting selenium species to selenate ion [Se(VI)] by heating with hydrogen peroxide and potassium permanganate and determination of the Se(VI) by ion chromatography (IC). However, this method was not applicable to the determination of selenium in sulphur-containing drugs because of the large amounts of sulphate formed by oxygen flask combustion and oxidation with hydrogen peroxide.

In this work, all selenium species formed by oxygen flask combustion were oxidized to Se(VI) by heating with nitric acid and potassium permanganate and the

resulting Se(VI) was determined by recycle IC to eliminate interfering ions. Recycle IC was used by Hoover and Yager^{6,7} to determine Se(IV), Se(VI) and arsenate in water. We have constructed a simpler recycle IC system that is easier to operate.

EXPERIMENTAL

Reagents

Deionized water (Millipore RO-Q system) was used throughout. Stock solutions of Se(IV) and Se(VI) (1.0 mg/ml as selenium) were prepared by dissolving 331.69 mg of sodium selenite pentahydrate (Merck, analytical-reagent grade) and 238.83 mg of sodium selenate (Nakarai Chemicals, extra pure grade), respectively, in 100 ml of water. A stock solution of elemental selenium [Se(0)] (*ca.* 50 mg/ml in carbon disulphide) was prepared as reported previously⁵. The chemicals tested were acetazolamide (Sigma), chlorothiazide (Sigma), *p,p'*-diaminodiphenyl sulphone (dapson; Tokyo Kasei Kogyo), dichlorophenamide (Sigma), hydroflumethiazide (Sigma), 2-mercapto-1-methylimidazole (methimazole; Nakarai Chemicals, extra pure grade) and prednisolone (Nakarai Chemicals, analytical-reagent grade). Other reagents used were of analytical-reagent grade.

Recycle IC conditions

Fig. 1 illustrates the recycle ion chromatograph system (Dionex 2010i) equipped with a sample loop (50 μ l), an HPIC-TAC-1 concentration column, an HPIC-AG4 guard column (50 \times 4 mm), an HPIC-AS4 separation column (250 \times 4 mm), an AFS-1 packed hollow-fibre suppressor and a conductivity detector.

A sample solution was injected into the sample loop and a fraction of eluate containing Se(VI) was collected on a concentrator (C_1) by switching a valve (V_1). Switching times were defined in advance according to the retention time of Se(VI) (standard solutions for the calibration graph). This column and connection were washed with 2 ml of water through the injection port (I_2) using a disposable syringe. The collected Se(VI) was reinjected into the separation column by switching a valve (V_2).

The eluent selected was 4 mM sodium carbonate–4 mM sodium hydrogen-carbonate and the flow-rate was 1.5 ml/min. The scavenger for the suppressor was 50 mM dodecylbenzenesulphonic acid (DBS) (Tokyo Kasei Kogyo) at a flow-rate of 2.0 ml/min.

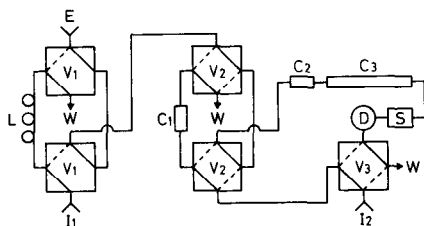


Fig. 1. Schematic diagram of recycle ion chromatography system. (C_1) Concentration column; (C_2) guard column; (C_3) separation column; (D) conductivity detector; (E) eluent in; (I_1 , I_2) injection ports; (L) sample loop; (S) suppressor; (V_1 – V_3) air-operated valves; (W) waste.

Preparation of samples

Each sample (50 mg) was weighed on a filter-paper (Toyo Roshi, No. 51A) and decomposed by the oxygen flask combustion method¹. The oxygen flask used was a glass-stoppered 500-ml hard glass flask with a platinum basket and the absorbing liquid was 10 ml of 0.7% nitric acid. The flask was rinsed with 10 ml of water and the washings were combined with the contents of flask. The solution was boiled and concentrated to about 1 ml. To the solution were added dropwise 20 mM potassium permanganate until a purple colour remained. One more drop of 20 mM potassium permanganate was added to the mixture and the mixture was evaporated to dryness on a hot-plate (140°C). To the residue were added 10 ml of water. The mixture was filtered through a 0.45- μ m membrane filter (Nihon Millipore Kogyo, SJHV 013 NS) and subjected to IC. Concentrations of selenium in the samples were calculated from a calibration graph of peak height *versus* concentration.

RESULTS AND DISCUSSION

Recycle IC conditions

The recycle system of Hoover and Yager^{6,7} was equipped with a suppressor column but we employed a continuously usable fibre suppressor. Generally, sulphuric acid is used as a scavenger for the fibre suppressor⁸⁻¹⁰, but some of the sulphate ions leaked from the membrane of the fibre suppressor, were collected on the concentrator and gave a large system peak of sulphate ion on reinjection (Fig. 2A). The use of DBS as a scavenger was recommended by Hanaoka *et al.*¹¹. Although it also gave system peaks due to sulphite and sulphate ions (Fig. 2B and C), neither of these ions interfered seriously with the determination of Se(VI). Therefore, DBS was selected as a scavenger.

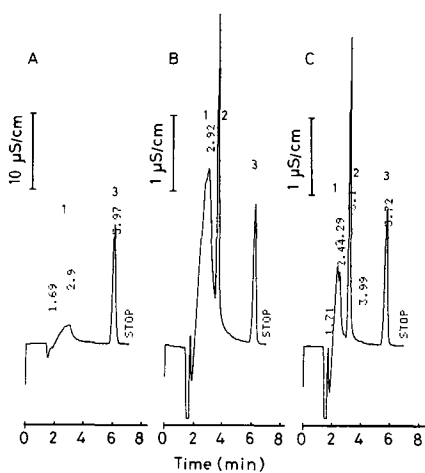


Fig. 2. System peaks in recycle ion chromatography. Scavenger: (A) 12.5 mM sulphuric acid; (B) 50 mM DBS; (C) DBS and the concentrator was washed with 2 ml of water before reinjection. The flow-rate of the scavengers was 2.0 ml/min and the recycle volume was 1.5 ml (1 min). Peaks: 1 = carbonate; 2 = sulphite; 3 = sulphate.

TABLE I

PERMISSIBLE CONCENTRATIONS OF FOREIGN IONS IN THE DETERMINATION OF SELENATE BY ION CHROMATOGRAPHY

Method	Permissible concentration* (mM)			
	Chloride	Phosphate	Nitrate	Sulphate
Conventional IC	25	10	2.4	0.4
Recycle IC	200	100	50	10

* Concentration causing the peak height for 2 μM selenate to be in error by more than 5%.

Carbonate ion in the eluent was also collected on the concentrator and gave a broad tailing system peak (Fig. 2A and B). As in the method of Hoover and Yager⁶, carbonate ion was removed by washing the concentrator with 2 ml of water (Fig. 2C) after collection.

The same eluent as mentioned in the previous paper⁵, 4 mM sodium carbonate–4 mM sodium hydrogencarbonate, was also used in this study because it gave the highest sensitivity and reproducible elution for the determination of Se(VI) on an HPIC-AS4 column.

The peak height of Se(VI) determined by recycle IC was 95% of that determined by conventional IC throughout the determination range. The determination range of Se(VI) by recycle IC was 42 nM–26 μM with relative standard deviations (R.S.D.) of 1.42% (1 μM , $n = 10$) and 0.61% (10 μM , $n = 10$). The proposed method permitted the coexistence of other anions at concentrations 10–25 times higher than in conventional IC, as shown in Table I. Fig. 3 shows typical chromatograms of Se(VI) in the presence of sulphate ion before and after recycling.

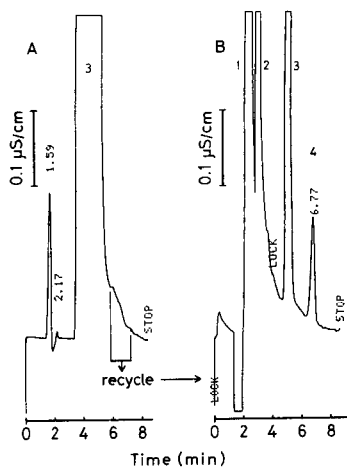


Fig. 3. Effect of recycle ion chromatography. Chromatograms (A) before and (B) after recycling. Sample: 380 nM Se(VI) in 10 mM sulphate. Peaks: 1 = carbonate; 2 = sulphite; 3 = sulphate; 4 = selenate.

Oxidation conditions for Se(0) and Se(IV)

Selenium was converted to Se(0), Se(IV) and Se(VI) (in the ratio 5:75:20) by oxygen flask combustion as described previously⁵. Since Se(VI) was determined most sensitively and was well separated from other ions by IC⁵, oxidation conditions for Se(0) and Se(IV) were examined.

All drugs to which the JP XI¹ and USP XXI² methods for the determination of selenium are applicable contain sulphur, except prednisolone. The sulphur was converted into sulphite and sulphate ions (in the ratio 80:20) by oxygen flask combustion. In our previous work⁵, all of the sulphite was oxidized to sulphate by heating with hydrogen peroxide, and the sulphate ion obtained interfered with the determination of Se(VI) by IC. Therefore, we used nitric acid, which is generally used to oxidize Se(0)^{1,2}. The sulphite was volatilized as sulphur dioxide by heating with nitric acid, and the recoveries of sulphur as sulphate were 15–25%. Further, nitrate ion was removed to a tolerable level for IC by heating to dryness.

However, when Se(0) was heated to dryness with nitric acid, Se(0) was oxidized to Se(IV) (recovery 40%) and Se(VI) (recovery 30%), and losses of 30% were observed. The losses are probably caused by volatilization of selenium dioxide formed from Se(IV). Therefore, Se(IV) was oxidized to Se(VI) by heating with potassium permanganate before the sample solution had been completely dried. Se(0) was oxidized to Se(VI) by heating with 0.1 ml of 70% nitric acid and 50 μ l of 20 mM potassium permanganate solution (Fig. 4). The heating temperature was 140°C, because the recoveries of Se(VI) on heating at 100–140°C were nearly 100% whereas heating above 160°C reduced the recovery. The recovery of selenium was 99.4% with an R.S.D. of 0.86% [$n = 8$, 127-nmol sample of Se(0) (12.7 μ M)].

Determination of selenium in drugs

When the above method was applied to analysis of drugs for selenium after oxygen flask combustion, reducing agents (not only sulphite but also halide and nitrite) were formed from many drug molecules. These reducing agents immediately consumed permanganate before the oxidation of Se(IV) had been completed. Therefore, the absorbing liquid obtained by oxygen flask combustion was boiled and concentrated to about 1 ml to volatilize the reducing agents. Then 20 mM potassium permanganate solution was added dropwise until the purple colour of permanganate remained, and one more drop was added. The times required for evaporation after

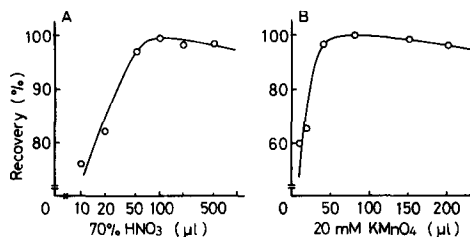


Fig. 4. Effect of reagent volume on the oxidation of Se(0) (127 nmol in 10 ml). (A) 50 μ l of 20 mM potassium permanganate added; (B) 100 μ l of 70% nitric acid added. The resulting Se(VI) was determined by conventional IC.

TABLE II
RECOVERY OF SELENIUM IN DRUGS BY ION CHROMATOGRAPHY

Sample (50 mg)	Se(0) (nmol)		Recovery (%)	R.S.D. (%)	n
	Added	Found			
Acetazolamide	0.0	0.0	—	0	4
	19.0	18.21	95.8	3.80	4
Chlorothiazide	0.0	0.0	—	0	4
	19.0	18.26	96.1	4.33	4
Dapsone	0.0	0.69	—	15.3	4
	19.0	19.86	100.9	2.70	4
Dichlorophenamide	0.0	0.0	—	0	4
	19.0	18.96	99.8	1.68	4
Hydroflumethiazide	0.0	0.0	—	0	4
	19.0	18.33	96.5	0.61	4
Methimazole	0.0	1.23	—	12.8	4
	6.33	7.54	99.8	4.61	8
	37.97	40.27	102.8	1.91	8
Prednisolone	0.0	0.0	—	0	4
	6.33	6.35	100.4	3.04	8
	37.97	36.04	94.9	5.69	5

addition of potassium permanganate were about 5 min, while the oxidation of Se(IV) was completed within 1–2 min.

Table II shows the recovery of selenium added to several drugs. Except for prednisolone, which could be analysed by the conventional IC, these drugs were analysed by recycle IC. The recoveries were in the range 95–103% with an R.S.D. of 1–6% in each instance.

The overall procedure was completed within 1.5 h. As oxygen flask combustion prevents the loss of analyte and is more rapid than wet digestion, and recycle IC permits the coexistence of many more substances than in fluorimetry and HG-AAS, this method is useful for the determination of trace amounts of selenium in organic compounds.

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