Determination of Selenium in Bulk Prednisolone by Ion Chromatography

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A method for the determination of selenium by ion chromatography (IC) after oxygen flask combustion has been developed and applied to the determination of selenium in bulk prednisolone. We found elemental selenium [Se(0)], selenite ion [Se(IV)] and selenate ion [Se(VI)] (ca. 5, 75 and 20%) in the absorbing liquid obtained by oxygen flask combustion of Se(0), Se(IV) and Se(VI). Se(VI) was directly determined by IC. Se(IV) was determined as Se(VI) after oxidation by potassium permanganate. Se(0) was oxidized to Se(VI) by hydrogen peroxide and potassium permanganate. In this method, the recovery of selenium was in the range of 95–100%, and the detection limit was 63 ng (79 pg with concentrator) in 40 mg of sample.

Keywords: Selenium, oxygen flask combustion, ion chromatography, prednisolone

Experimental

Reagents
Stock solutions of Se(IV) and Se(VI) were prepared by dissolving 331.69 mg of sodium selenite pentahydrate (Merck, reagent grade) and 238.83 mg of sodium selenate (Nakarai Chemicals, extra pure) in 100 ml of water.

The stock solution of Se(0) (ca. 50 mg/1 in carbon disulfide) was prepared as follows. A 20 mg portion of sodium selenite pentahydrate was dissolved in 20 ml of water, and 0.5 g of magnesium powder (Kanto Chemical, extra pure) was added. To this solution was added 5 ml of hydrochloric acid during cooling in an ice-water bath. After the magnesium powder was dissolved, Se(0) was extracted with 100 ml of carbon disulfide; the system was standardized by DAN-fluorometry.4

Prednisolone from Nakarai Chemicals, reagent grade, was used. Other reagents used were of reagent grade. Deionized water (Millipore RO-Q system) was used throughout the present study.

Apparatus
The ion chromatograph used was Dionex 2010i equipped with a sample loop (50 µl), a HPIC-TAC-1 concentrator column, a HPIC-AG4 guard column (5 cm×4 mm i.d.), a HPIC-AS4 separator column (25 cm×4 mm i.d.), a packed hollow fiber suppressor and a conductivity detector. The fluorospectrometer used was a Shimadzu RF-510 spectrofluorometer. The oxygen flask used was a glass stoppered 500-ml hard glass flask with a platinum basket.

IC conditions
The eluent selected was 4 mM sodium carbonate-
4 mM sodium bicarbonate and the flow rate was 1.5 ml/min. The scavenger for the suppressor was 12.5 mM sulfuric acid at a flow rate of 2.0 ml/min.

**Ion chromatographic determination**

Each sample (40 mg) was weighed on a filter paper (Toyo Roshi, No. 51A) and decomposed by the oxygen flask combustion method (absorbing liquid was 10 ml of water). The flask was rinsed with 10 ml of 30% hydrogen peroxide, and the washings were combined with the content of the flask in a 50-ml beaker. The solution was boiled gently for 20 min, with a watch glass covering. The solution was added with a few drops of 20 mM potassium permanganate until gas evolution; the mixture was concentrated to 10 ml after completing the gas evolution. To the solution were added 100 µl of 0.1 M hydrochloric acid and 50 µl of 20 mM potassium permanganate. The mixture was boiled for 5 min, and then 50 µl of 3% hydrogen peroxide was added. After cooling water was added to make up the volume to 20 ml. The resulting solution was filtered through a 0.45 µm membrane filter (Nihon Millipore Kogyo K.K., SJHV 013 NS), and subjected to the chromatograph. Concentrations of Se(VI) in unknown samples were calculated from the peak heights of calibration curves made for diluted stock solutions of Se(VI).

**Results and Discussion**

**IC conditions**

Carbonate-bicarbonate eluent has been used widely with AS-4 column for separation of common anions. The eluent, 4 mM sodium carbonate-4 mM sodium bicarbonate, at a flow rate of 1.5 ml/min presented rapid, reproducible and most sensitive elution of Se(VI), as shown in Table 1. This condition gave a satisfactory resolution of Se(VI) from sulfate ion, which interfered most severely with the determination of Se(VI). Figure 1 shows the simultaneous separation of Se(IV), Se(VI) and other anions. The peak height of Se(IV) was 80% of Se(VI) in equimolar concentration. Se(VI) was separated well from other ions, while Se(IV) was affected by carbonate, chloride and phosphate, since those were eluted close to the peak of Se(IV). Base line drift caused by water dip also interfered with the determination of Se(IV). Therefore it was difficult to determine less than 0.5 µM of Se(IV).

The calibration curve of Se(VI) was linear from 0 to 25 µM, with relative standard deviations (RSD) of 3.93% (0.1 µM, n=10) and 0.35% (10 µM, n=10). The lower detection limit (S/N=3) was 40 nM (3.2 ppb, as selenium) with a 50 µl loop injector, and 50 pM (4.0 ppt, as selenium) with a concentrator column (load volume was 50 ml).

**Valence state of selenium produced by oxygen flask combustion**

When any of Se(0), Se(IV) or Se(VI) were decomposed with prednisolone by the oxygen flask combustion method according to the method of JP XI, we found not only Se(IV) but Se(VI) in the absorbing liquid. Furthermore, the presence of Se(0) in the absorbing liquid was confirmed experimentally by later measures.

![Fig. 1 Separation of anions. Eluent, 4 mM Na₂CO₃-4 mM NaHCO₃; flow rate, 1.5 ml/min; (a) 25 µM chloride; (b) 50 µM selenite; (c) 59 µM bromide; (d) 100 µM nitrate; (e) 25 µM sulfate; (f) 50 µM selenate.](image)

<table>
<thead>
<tr>
<th>Eluent/mM</th>
<th>Flow rate/ ml min⁻¹</th>
<th>tₑ/ min</th>
<th>Rₑ</th>
<th>LOD/ nM</th>
<th>RSD / %</th>
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</table>

a. Retention time. b. Resolution between sulfate and Se(VI). c. Lower limit of detection. d. Sample was 280 nM Se(VI), n=10.
investigation, as follows: To the filter paper was absorbed 25.3 µl of 10 mM sodium selenite (as selenium 20 µg); then this filter paper was decomposed by oxygen flask combustion. To the absorbing liquid was added 10 ml of carbon disulfide, and the mixture was shaken well. When carbon disulfide layer was evaporated, a dark-red precipitate (red selenium) appeared. The precipitate was dissolved in 1.0 ml of 35% nitric acid, and determined by DAN-fluorometry. Then we found 0.7–1.2 µg of selenium (ca. 5% of added selenium).

Selenium can be determined as Se(IV) by DAN-fluorometry or as Se(VI) by IC as described above. To conform the valences of all selenium species to Se(IV), oxidation of Se(0) and reduction of Se(VI) are required. Therefore the procedure may need many steps and take a long time. On the other hand, Se(0) and Se(VI) are conformed to Se(VI) by only oxidation. Thus oxidation conditions of Se(0) and Se(IV) were examined.

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

Generally, oxidation of Se(0) is carried out by heating it with nitric acid and/or perchloric acid. However, these reagents are unusable for IC because of interferences caused by large amounts of acids in the resulting mixture. Hydrogen peroxide is an oxidizing reagent for selenium and theoretically forms only water, which does not interfere with the ion chromatographic determination. Thus the utility of hydrogen peroxide was examined. As shown in Fig. 2, Se(0) was oxidized quantitatively by boiling with 15% hydrogen peroxide for 20 min. In this step, Se(0) was oxidized to Se(VI) and Se(IV) in 20% and 80% yield, respectively.

Se(IV) was oxidized by either potassium permanganate in hydrochloric acid solution or hypochlorite (potassium or sodium salt). The oxidizing method using potassium permanganate gave fewer and smaller interfering peaks in the chromatogram, and potassium permanganate was useful to decompose the hydrogen peroxide which had been used to oxidize Se(0). Therefore, we used potassium permanganate for conversion of Se(IV) to Se(VI). Se(IV) was oxidized to Se(VI) by boiling with 0.1 mM potassium permanganate and 1.0 mM hydrochloric acid for 5 min, as shown in Fig. 3.

**Determination of selenium in bulk prednisolone**

As shown in Fig. 4, Se(VI) was well separated from other components which resulted from oxygen flask combustion and oxidations by hydrogen peroxide and potassium permanganate in hydrochloric acid solution. Table 2 shows recoveries of total selenium and each selenium species in the absorbing liquid obtained by oxygen flask combustion of Se(0) added to predni-

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**Fig. 2** Effect of H$_2$O$_2$ concentration and boiling time on oxidation of selenium. Sample, Se(0) 50.6 nmol/10 ml. H$_2$O$_2$ concentration: (○) 20%; (□) 15%; (△) 10%.

**Fig. 3** Effect of HCl and KMnO$_4$ concentrations on oxidation of selenite. Sample, Se(IV) 63.3 nmol/10 ml. HCl concentration: (○) 1 mM; (□) 0.1 mM; (△) 0.01 mM; (△) 0 mM.

**Fig. 4** Chromatogram of selenium added to prednisolone treated with oxygen flask combustion and oxidations by H$_2$O$_2$ and KMnO$_4$. Sample, Se(0) 25.3 nmol added to 40 mg of prednisolone.
Table 2 Recovery of selenium added to prednisolone

<table>
<thead>
<tr>
<th>Sample/mg</th>
<th>Se(0)/nmol</th>
<th>Recovery, %</th>
<th>Ratio of product, %</th>
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<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
<td>[RSD*,%]</td>
</tr>
<tr>
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<tr>
<td>40</td>
<td>56</td>
<td>53.75</td>
<td>[0.67]</td>
</tr>
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</table>

a. n=4.

The original amount of Se(VI) in the absorbing liquid was determined directly. The amount of Se(IV) was calculated from the amounts of Se(VI) determined before and after oxidation by potassium permanganate in hydrochloric acid solution. The amount of Se(0) was also calculated from the amount of Se(VI) determined after oxidation by potassium permanganate in hydrochloric acid solution which could not oxidize Se(0), and that obtained after oxidation by hydrogen peroxide and potassium permanganate in hydrochloric acid solution. Se(0), Se(IV) and Se(VI) were produced in the ratio 5:75:20. The recoveries of Se(IV) alone, and of Se(IV) plus Se(VI) obtained here well agreed with those reported in the previous paper\(^4\), in which direct DAN-fluorometry (76.8% recovery) and the DAN-fluorometry after reduction of Se(VI) by hydrochloric acid (92.6% recovery) were examined.

In addition to prednisolone, thiamazole was also examined to determine selenium contaminant. It was impossible to determine selenium in thiamazole by IC because of the large amounts of sulfate formed from sulfur in thiamazole molecule by oxygen flask combustion and oxidations by hydrogen peroxide and potassium permanganate. We are investigating a method to determine selenium in thiamazole.

References


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