Inorganic Chemical Approaches to Pharmacognosy. VIII.[1] Determination of Selenium in Crude Drugs by Energy-Dispersive X-Ray Fluorescence Spectrometry

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Energy-dispersive X-ray fluorescence spectrometry following sample decomposition and preconcentration was developed to determine selenium in crude drugs. Samples were decomposed with conc. HNO_3 and conc. H_2SO_4 in a flask with a reflux condenser. Evaporation of HNO_3 had to be avoided to prevent serious loss of this element. Selenium was preconcentrated from the digestion liquid by two-step reduction with 4 M HCl and ascorbic acid, the elemental selenium formed was adsorbed on activated carbon and then collected on a Nuclepore membrane filter for direct irradiation in an X-ray spectrometer.

This analytical method (detection limit, 0.03 ppm) was used to determine selenium in many kinds of crude drugs. The analytical results indicated vegetable drugs to be low in selenium content: more than 0.5 ppm in only a limited number of samples and less than detection limit (0.03 ppm) in nearly all the samples. Animal drugs contain selenium at higher levels, Lumbricus and Cantharis being 7.46 and 1.67 ppm, respectively.

Keywords selenium; crude drug; energy-dispersive X-ray fluorescence spectrometry; determination

Selenium is necessary for proper growth and fertility in animals and deficiency in animal diets leads to serious disease, most of which being due to inadequate vitamin E. At the same time, selenium levels slightly above the nutritional requirement are highly toxic and the permissible intake level has been found to range from 0.5 to 3.5 ppm in feed.[2,3] Selenium, far from being the carcinogenic agent that some early experiments had appeared to suggest, may actually be a cancer-protecting element.[4] In spite of the considerable biological and environmental importance of selenium, no data are presently available on its content in crude drugs. Thus, in the course of our studies to elucidate the significance of inorganic component in crude drugs, this parameter was examined for various crude drugs.

The techniques most commonly used for selenium determination are neutron activation analysis, fluorometry, and atomic absorption spectrometry. The first is highly sensitive but not widely used because of the expense and time required. The last two incur interference by concomitant ions, and the procedures are quite complex. X-Ray fluorescence spectrometry (XRF) is a convenient analytical method and suitable for many samples containing selenium at relatively high concentrations. Even for low-selenium samples with less than the typical detection limit (1—2 ppm), XRF conducted with the proper preconcentration should permit analysis at sufficient sensitivity.

This paper reports a relatively simple and reliable analytical method for selenium in about 75 crude drugs by XRF with a preconcentration such that the elemental selenium reductively formed is adsorbed onto an activated carbon.

Experimental

Apparatus Intensity measurements were made on a Rigaku-Kevex energy-dispersive X-ray spectrometer (ultra-trace system), including a molybdenum anode X-ray tube, secondary molybdnum target, and a filter assembly to generate bichromatic radiation, a $30\,mm^2 \times 3\,mm$ Si (Li) detector, an X-ray amplifier, and a conventional multi-channel analyzer. The X-ray tube was operated at 40 kV and 40 mA, and accumulation time was 2000 s. Selenium analysis was conducted with the Kα line at 11.21 keV.

The filtration unit was comprised of a 300 ml glass filter funnel, a Millipore filter holder (XX10 047 00) with a 47 mm diameter (effective area: $9.6\,mm^2$) and a 47 mm diameter, 0.4 μm pore size Nuclepore membrane filter.

Materials The crude drug samples were kindly provided by Mikuni Co., Ltd., Koshiro Chuji Co., Ltd., Nihon Funmatsu Co., Ltd., and Shinwa Bussan Co., Ltd. (Osaka). The acid washed activated carbon (Norit A) was purchased from Nakarai Chemicals Ltd. All other reagents were of the highest quality available.

Preparation of Calibration Curve Transfer 0, 0.4, 0.8, 1.2, 1.6, and 2.0 ml aliquots of standard selenium solution (1 ppm) and 0.4 0.6, 0.8, and 1.0 ml of aliquots of standard selenium solution (10 ppm) into 150 ml flat bottom flasks. Add 15 ml of conc. HNO_3 and 5 ml of conc. H_2SO_4 to each. Add 100 ml of 4.8 M HCl (resulting solution is 4.0 M HCl), and allow it to stand for 30 min at 100 °C. After cooling, dilute with water to a final volume of 300 ml, and add 100 mg of activated carbon and 3.0 g of ascorbic acid. Stand for 2 h, stirring occasionally, and filtrate the active carbon with a 0.4 μm pore size 47 mm diameter Nuclepore membrane, followed by washing with *ca.* 100 ml of water. After drying, the filter is measured by a X-ray fluorescence spectrometer as described above.

Recommended Procedure for Sample Analysis Transfer a powdered sample (2.0 g) to a 150 ml flat bottom flask, add 15 ml of nitric acid, cover with a watch glass, and allow to stand overnight. After adding 5 ml of conc. H_2SO_4, heat the flask equipped with a reflux condenser in a sand bath to destroy all organic matter.[5] After cooling, if necessary, dilute with water to 20 ml, add 100 ml of 4.8 M HCl, and continue as in the preparation of the calibration curve.

Procedure for Thiourea–H_2SO_4 System A proper amount of selenium is transferred to a 150 ml flask containing 95 ml water and 5 ml conc. H_2SO_4, and 100 mg of activated carbon and 100 mg of thiourea are added. The resulting suspension is heated to boiling and refluxed for about 30 min. After cooling, the activated carbon with the adsorbed elemental selenium is filtered off on the same membrane as in the 4 M HCl–ascorbic acid system.

Results and Discussion

General Considerations Multi-elemental analyses (P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Rb, Sr, Pb, Mo, I, and Ba) were conducted on various crude drugs by the convenient XRF method as reported previously.[6] Important data were obtained on the characteristic metals profile of each crude drug and the relationship of this profile to the plant from which the drug came.[1,7-10] However, selenium in nearly all the samples examined could not be detected due to the low sensitivity (detection limit, *ca.* 1 ppm) of the method used. Selenium determination thus requires sample decomposition and preconcentration.

Heating is a most convenient ashing method but may involve loss of selenium. Wet oxidation with a suitable reagent is generally considered a very reliable ashing method. A $HNO_3+H_2O_2$, HNO_3+HClO_3, or $HNO_3+H_2SO_4$ system is frequently used as an oxidation reagent, but the use of H_2O_2 or $HClO_3$ needs strict caution to avoid the explosion of these reagents at heating. Taking into account both convenience and safety, we chose the conc. HNO_3–conc. H_2SO_4 system for oxidation. In recent years, selenium determination in environmental waters by XRF has been developed.[11] It is based on the reduction of dissolved selenate and selenite to elemental selenium by thiourea in H_2SO_4, which is subsequently adsorbed onto activated carbon to achieve an appropriate preconcentration. More recently, the reduction to elemental selenium by the HCl–ascorbic acid system, followed by XRF, has also been reported.[12] Thus, we used these methods for solutions after wet decomposition with conc. HNO_3 and conc. H_2SO_4.

Loss of Selenium during Wet Digestion with Conc. HNO_3 and Conc. H_2SO_4 The ashing method is of critical importance for selenium determination in consideration of its tendency to vaporize. To test for loss of selenium during analytical procedures involving wet oxidation with these acids, 1 μg of selenium with or without plant sample (2.0 g of Astragali Radix) was analyzed under various conditions. The results are given in Table I. When a standard solution of 1 μg selenium without a plant sample was analyzed, no loss was observed during ashing. However, the addition of the plant sample led to serious reduction (*ca.* 90%) of the analyte line intensity (fluorescent X-rays of Se Kα). The selenium added after evaporation of HNO_3 was recovered completely, suggesting a considerable decrease in the fluorescent X-ray intensity due to loss of selenium during ashing, especially the evaporation of HNO_3, rather than physical or chemical interference by inorganic elements from the plant sample with X-ray intensity. In fact, the modified digestion method, without the evaporation of HNO_3, gave satisfactory results.

TABLE I. Loss of Selenium during Wet Digestion with Conc. HNO_3 and Conc. H_2SO_4

Material (g)	Amount of Se added (μg)	Wet digestion	Intensity counts (%)
—	+Se (1)	−	2200 (100)
—	+Se (1)	+	2300 (104)
Astragali Radix (2.0)	+Se (1)	+	280 (13)
Astragali Radix (2.0)	0	+	100 (5)
Astragali Radix (2.0)	+Se (1)	+[a)]	2200 (100)
Astragali Radix (2.0)	+Se (1)	+[b)]	2200 (100)

a) One μg of selenium was added after the evaporation step of HNO_3. *b)* The evaporation step of HNO_3 was omitted in 4 M HCl–ascorbic acid system. (−), wet digestion was not performed; (+), wet digestion was performed.

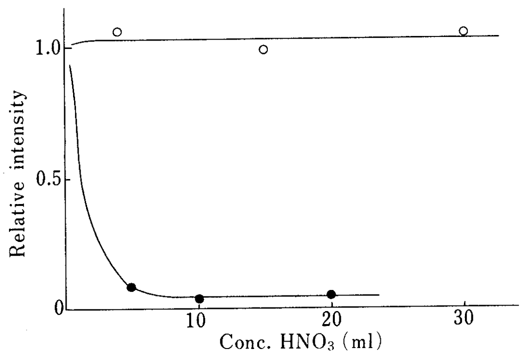

Fig. 1. Effect of HNO_3 on Intensity of Fluorescent X-Rays (Se Kα)
○, 4 M HCl–ascorbic acid; ●, thiourea in H_2SO_4.

Because the evaporation of HNO_3 was omitted, the effect of HNO_3 on the fluorescent X-ray intensity (Se Kα) had to be examined in both reduction procedures. As seen in Fig. 1, the fluorescent X-ray intensity of selenium was independent of the addition of HNO_3 in 4 M HCl–ascorbic acid system, while in the thiourea–H_2SO_4 system, X-ray intensity remarkably decreased according to the amount of HNO_3 added. The addition of 5 ml of HNO_3 gave about a 90% decrease in the intensity of the fluorescent X-rays. Since there is no interference by HNO_3, the 4 M HCl–ascorbic acid system is undoubtedly preferable as a reduction method from dissolved selenate or selenite to elemental selenium. Figure 2 shows the effects of ascorbic acid on the fluorescent X-ray intensity. More than 1.0 g of ascorbic acid gave unvariable intensity of the X-rays. Thus, 3.0 g of ascorbic acid were used as the amount to be added. Also, 100 mg of activated carbon were selected, taking into account the reproducibility and earlier recommendation,[11] although a lesser amount of activated carbon was expected to enhance analytical sensitivity.

Procedures and Calibration Curve Based on the above results, a recommended procedure (described in Experimental) was established for the determination of selenium in crude drugs by XRF.

The calibration curve for analysis of selenium in crude drugs is shown in Fig. 3. The detection limit (3σ) for selenium was 0.03 ppm (concentration in sample) and the standard deviation for a 1.0 ppm sample (2.0 g of Platycodi Radix with addition of 2 μg of selenium) was 5.8 % for 8 consecutive measurements.

Analytical Results Many kinds of crude drugs (75 in

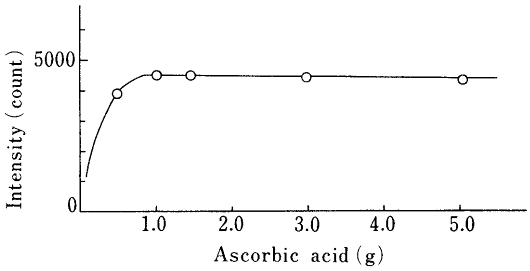

Fig. 2. Effect of Ascorbic Acid on Intensity of Fluorescent X-Rays (Se Kα)

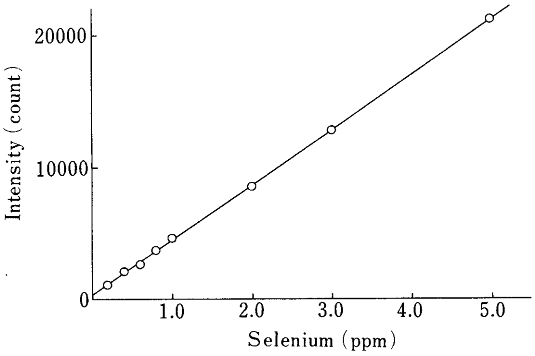

Fig. 3. Calibration Curve for Selenium in X-Ray Fluorescence Spectrometry after Sample Decomposition and Preconcentration

TABLE II. Analytical Results for Selenium in Various Crude Drugs

Material	Sample number	Selenium (ppm)
Cortex		
Cascara Sagrada (Rhamnaceae)	1	<0.03
Cinchonae Cortex (Rubiaceae)	1	<0.03
Cinnamomi Cortex (Lauraceae)	1	<0.03
Galla Rhois (Anacardiaceae)	1	<0.03
Granati Cortex (Punicaceae)	1	<0.03
Phellodendri Cortex (Rutaceae)	1	<0.03
Radix		
Aconiti Tuber (Ranunculaceae)	1	<0.03
Astragali Radix (Leguminosae)	2	<0.03
Bupleuri Radix (Umbellirerae)	1	<0.03
Gentianae Radix (Gentianaceae)	1	<0.03
Ginseng Radix (Araliaceae)	1	<0.03
Glycyrrhizae Radix (Leguminosae)	5	<0.03—0.10
Ipecacuanhae Radix (Rubiaceae)	1	<0.03
Platycodi Radix (Campanulaceae)	1	<0.03
Polygalae Radix (Polygalaceae)	1	<0.03
Scutellariae Radix (Labiatae)	1	<0.03
Senegae Radix (Polygalaceae)	1	0.27
Sophorae Radix (Leguminosae)	1	<0.03
Rhizoma		
Atractylodis Rhizoma (Compositae)	1	<0.03
Coptidis Rhizoma (Ranunculaceae)	1	<0.03
Curcumae Rhizoma (Zingiberaceae)	1	<0.03
Nupharis Rhizoma (Nymphaeae)	1	<0.03
Pinelliae Tuber (Araceae)	1	0.07
Rhei Rhizoma (Polygonaceae)	1	<0.03
Scopoliae Rhizoma (Solanaceae)	1	<0.03
Zingiberis Rhizoma (Zingiberaceae)	2	<0.03—0.51
Folium		
Daturae Folium (Solanaceae)	1	<0.03
Digitalis (Scrophulariaceae)	1	<0.03
Eucalypti Folium (Myrtaceae)	1	0.08
Menthae Folium (Labiatae)	1	<0.03
Sennae Folium (Leguminosae)	1	<0.03
Steviae Folium (Compositae)	1	0.11
Theae Folium (Theaceae)	1	<0.03
Uvae Ursi Folium (Ericaceae)	1	<0.03
Vitis-Idaeae Folium (Ericaceae)	1	<0.03
Flos		
Caryophylli Flos (Myrtaceae)	1	<0.03
Chamomillae Flos (Compositae)	1	<0.03
Crocus (Iridaceae)	1	<0.03
Santonica (Compositae)	1	<0.03
Sophorae Flos (Leguminosae)	1	<0.03
Fructus		
Aurantii Pericarpium (Rutaceae)	1	<0.03
Cardamomi Fructus (Zingiberaceae)	1	0.09
Foeniculi Fructus (Umbelliferae)	1	<0.03
Kamala (Euphorbiaceae)	1	<0.03
Piperis Nigri Fructus (Piperaceae)	1	<0.03
Zanthoxyli Fructus (Rutaceae)	1	<0.03
Semen		
Arecae Semen (Palmae)	1	<0.03
Armeniacae Semen (Cruciferae)	1	<0.03
Cassiae Semen (Leguminosae)	1	0.39
Coffeae Semen (Rubiaceae)	1	<0.03
Coicis Semen (Gramineae)	1	<0.03
Hydnocarpi Semen (Flacourtiaceae)	1	<0.03
Lini Semen (Linaceae)	2	0.65—1.33
Myristicae Semen (Myristicaceae)	1	<0.03
Pharbitidis Semen (Convolvulaceae)	1	<0.03
Ricini Semen (Euphorbiaceae)	1	<0.03
Sinapis Semen (Cruciferae)	2	<0.03—0.54
Strychni Semen (Loganiaceae)	1	<0.03
Theobromatis Semen (Sterculiaceae)	1	0.04
Tiglii Semen (Euphorbiaceae)	1	<0.03
Others		
Aloe (Liliaceae)	1	<0.03
Digenea (Rhodomelaceae)	1	0.11
Ephedrae Herba (Ephedraceae)	1	<0.03
Gambir (Rubiaceae)	1	<0.03
Lycopodium (Lycopodiaceae)	1	<0.03
Materia medica animalis		
Bufonis Venenum (Bufonidae)	1	0.15
Cantharis (Meloidae)	1	1.67
Cervi Parvum Cornu (Cervidae)	1	0.19
Coccionella (Coccidae)	1	<0.03
Eupolyphaga (Blattidae)	1	0.33
Gecko (Gekkonidae)	1	0.57
Lacca (Coccidae)	1	<0.03
Lumbricus (Megascolecide)	3	2.81—7.46
Hirudo (Hirudidae)	1	0.32
Scorpion (Buthidae)	1	0.99

number) were analyzed by the above procedure. The results are summarized in Table II. The three highest values in all vegetable drugs examined were 1.33 for Lini Semen, 0.54 for Sinapis Semen, and 0.51 ppm for Zingiberis Rhizoma, with nearly all the other vegetable drugs being less than the detection limit (0.03 ppm). The three highest values in all animal drugs were 7.46 for Lumbricus, 1.67 for Cantharis, and 0.99 ppm for Scorpion. Animal drugs appear to contain selenium at obviously higher levels than vegatable drugs.

In general foods, most fruits and vagetables contained quantities of selenium less than 0.01 ppm. The exceptions were garlic, mushroom, and radish containing 0.25, 0.13, and 0.04 ppm, respectively. Meat samples ranged from about 0.1 ppm for chicken muscle to as high as 1.9 ppm for pork kidney with most values between 0.2 and 0.5 ppm. The content in seafood was generally higher, ranging from 0.4 to 0.7 ppm.[13] Our results for the vegetable drugs, less than 0.03—1.33 ppm (mean value is 0.09 or less[14]) are in reasonable agreement with those for general vegetables. The highest selenium content among the vegatable drugs tested was observed for Lini semen. This finding is interesting because linseed oil meal provides a protective effect against chronic selenium poisoning in rats.[15] Furthermore, we detected 0.51 ppm of selenium in Sinapis Semen, the second highest value among the vegetable drugs. Williams *et al.*[16] also reported 5 ppm of selenium in one sample among 23 samples of Sinapis Semen, and less than 3 ppm for others. This crude drug contains characteristic sulfur-containing compounds such as sinigrin. It is of interest in this regard that Allium species such as garlic and onion, which contain comparatively larger amount of organic sulfur compounds, are relative high in selenium.[17,18]

Finally, it is well known that relatively large quantities of selenium are present in certain species of Genus *Astragalus*, which are harmful to animals consuming these range plants.[19] But Astragali Radix, which probably originated from Astragalus menbranaceus, or *A. monghoicus*, contains only less than 0.03 ppm of selenium, suggesting no environmental problem although sample 2 was too small to conclude so.

Since we dealt with many kinds of crude drugs, samples for one kind of drug were limited. We plan to determine the selenium content of many specimens of one kind of drug.

Conclusions

For selenium determination in crude drugs, we developed a relatively simple and reliable XRF method in which sample decomposition is carried out using conc. HNO_3 and conc. H_2SO_4 as wet oxidation reagents and colloidal selenium reductively formed in the HCl–ascorbic acid system is collected on activated carbon. This is different from the method of Robberecht *et al.*[12] in which the explosive system of HNO_3–$HClO_3$ was used as an oxidation reagent and the evaporation of HNO_3 was required, and has the advantages of safety and convenience.

The analytical results for many crude drugs indicated vegetable drugs to contain selenium at lower concentrations than animal drugs. Selenium content in most vegatable drugs was less than the detection limit (0.03 ppm Se). The typical exceptions were Lini Semen, Sinapis Semen, and Zingiberis Rhizoma containing 1.33, 0.54, and 0.51, respectively. Almost all animal drugs were more than 0.03 ppm, the highest values being 7.46 and 1.67 ppm for Lumbricus and Cantharis, respectively. However, it should be emphasized that individual and geographical variation for one kind of crude drug remains to be investigated.

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