

# Determination of Al, Cr, Fe, Zn, Cd, Pb and Bi in Crude Drugs by Inductively Coupled Plasma Atomic Emission Spectrometry after Coprecipitation with Yttrium Phosphate

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A novel method that combines inductively coupled plasma atomic emission spectrometry (ICP-AES) with a coprecipitation technique to determine trace and sub-trace elements in crude drugs is described herein. Yttrium phosphate quantitatively coprecipitated seven elements (Al, Cr, Fe, Zn, Cd, Pb and Bi) at pH 6 in a solution prepared from microwave-digested samples of crude drugs. Some matrix elements such as Na, K, Mg and Ca were effectively removed by this process. The thus coprecipitated elements were then determined by ICP-AES with yttrium as an internal standard element. The detection limits ( $3\sigma$ ,  $n = 10$ ) were 0.17 mg/kg for Al, 0.004 mg/kg for Cr, 0.03 mg/kg for Fe, 0.13 mg/kg for Zn, 0.004 mg/kg for Cd, 0.19 mg/kg for Pb and 0.06 mg/kg for Bi. The proposed method was successfully utilized to determine the concentration of the above-mentioned seven elements in standard reference materials [National Institute of Standards Technology (NIST) SRM1515, SRM1547 and SRM1575a] and seven kinds of crude drugs.

**Key words** — crude drug, toxic element, coprecipitation, inductively coupled plasma atomic emission spectrometry, determination

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## INTRODUCTION

Crude drugs, which are unrefined substances derived from organic or inorganic sources such as plants, animals, organs, or minerals are widely used as raw materials for medicines or complementary or alternative synthetic medicines. Because they are naturally occurring, crude drugs occasionally contain some toxic elements such as Cd and Pb. Therefore, identifying the presence of toxic elements in crude drugs is important from the viewpoint of quality control and traceability. Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a convenient technique for determining the presence of elements in crude drugs because this technique can determine various elements simultaneously.<sup>1)</sup> However, the coexistence of large amounts of the matrix elements such as Na, K, Mg and Ca sometimes cause serious interference. Combining ICP-AES with a separation technique is a useful way to solve this problem. Among the various separation techniques that have been proposed so far,<sup>2,3)</sup> coprecipitation is a simple technique for separating elements. We previously reported that yttrium phosphate is an excellent coprecipitant for Pb, Fe and Bi.<sup>4,5)</sup> This coprecipitant not only removes Na, K, Mg and Ca but also aggregates the elements to be measured. In the present report, we utilized this coprecipitation technique to determine seven elements (Al, Cr, Fe, Zn, Cd, Pb and Bi) in crude drugs by ICP-AES.

## MATERIALS AND METHODS

**Apparatus** — An Optima 5300DV inductively coupled plasma atomic emission spectrometer (PerkinElmer Inc., Winter Street Waltham, MA, U.S.A.) was used to determine the elements, the emission intensities of which were measured in the axial (Al, Cr, Fe, Zn, Cd, Pb and Bi) and radial (Y) view modes. A Multiwave3000 microwave digestion system (Anton Paar GmbH, Graz, Austria) was used to digest the samples. The pH measurements were carried out using a Horiba model F-22 pH meter (HORIBA Ltd., Kyoto, Japan). In all of the experiments purified water (18.2 M $\Omega$ ) from a Milli-Q-system (Millipore, Billerica, MA, U.S.A.) was used. **Reagents** — A yttrium solution (10 g/l, 4% nitric acid) was purchased from SCP SCIENCE (Quebec, Canada). An ICP multi-element standard solution IV (Merck, Darmstadt, Germany), containing

**Table 1.** Analytical Wavelength, Range of Calibration Curve and Detection Limit

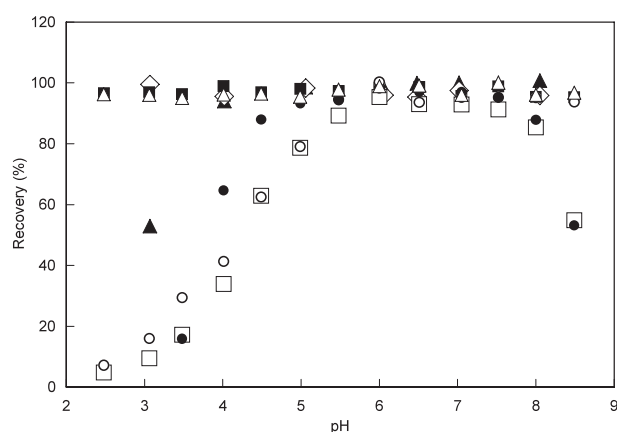
Element	Analytical wavelength (nm)	Range of Calibration Curve ( $\mu\text{g}$ ) <sup>a)</sup>	Detection Limit ( $\mu\text{g}$ ) <sup>a,b)</sup>
Al	396.153	0.5 – 100	0.17
Cr	205.560	0.025– 50	0.004
Fe	283.204	0.25 – 100	0.03
Zn	206.200	0.5 – 50	0.13
Cd	214.440	0.025– 50	0.004
Pb	220.353	0.5 – 50	0.19
Bi	190.171	0.2 – 50	0.06
Y	371.029 <sup>c)</sup>		

a) The amount in the solution after the coprecipitation. b)  $3\sigma$ ; Three times the standard deviations obtained from 10 replicate determinations of the blank. c) Internal standard element.

1000 mg/l of Al, Cr, Fe, Zn, Cd, Pb, Bi and the other elements, was used as a standard solution of elements. The other reagents used for this investigation were of guaranteed or analytical reagent grades.

**Samples**— Commercially available crude drugs with officially confirmed qualities in the Japanese Pharmacopoeia XV were used (Shibata & Co., Ltd., Tokyo, Japan). The samples were ground finely in a vibrating mill before use. Standard reference materials were also used to validate the proposed method. The samples were digested based on references;<sup>6,7)</sup> only concentrated nitric acid (6.0 ml) was used for the microwave digestion of the samples (0.5–1.0 g). The heating program consisted of the following steps: 0–150 W in 15 min and holding at 150 W for 10 min, 150–400 W in 15 min and holding at 400 W for 10 min, and 400–700 W in 15 min and holding at 700 W for 10 min. After the digestion, the solution was filtered through a Polytetrafluoroethylene (PTFE) membrane filter (ADVANTEC DISMIC-25HP, pore size of 0.20  $\mu\text{m}$ , Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The filtrate was placed in a 50 ml of centrifuge tube and then diluted with purified water to approximately 10 ml.

**Determination of Elements**— A total 0.5 ml of 10 g/l yttrium solution and 1 ml of 0.5 mol/l phosphoric acid solution were added to the sample solution. The pH of the solution was adjusted to 6.0 using aqueous ammonia and a pH meter. The resulting precipitate was then centrifuged at 3000 rpm for 5 min. After the supernatant solution was discarded by decantation, approximately 15 ml of purified water was added to the centrifuge tube and the tube was shaken vigorously by hand. The precipitate suspended in the solution was re-centrifuged, and the precipitate was then dissolved in 5 ml of approximately 1.6 mol/l nitric acid. The seven elements



**Fig. 1.** Effect of pH on the Recoveries of Seven Elements After Coprecipitation with Yttrium Phosphate

Sample, solution (10 ml) after digestion of 1.0 g of *Dioscoreae Rhizoma*; each element, 10  $\mu\text{g}$  for Cr (○), Zn (●), Cd (□), Pb (■) and Bi (△) or 100  $\mu\text{g}$  for Al (▲) and Fe (◇); yttrium, 5 mg; 0.5 mol/l phosphoric acid, 1 ml.

in the precipitate-dissolved solution were then determined by ICP-AES with yttrium as an internal standard element. The analytical wavelengths, the ranges of the calibration curves, and the detection limits for each element are summarized in Table 1.

## RESULTS AND DISCUSSION

### Optimum Conditions for Coprecipitation

After spiking a solution prepared from the microwave digestion of 1.0 g of *Dioscoreae Rhizoma* (a popular crude drug) with known amounts of the seven elements to be determined, the yttrium phosphate coprecipitation method was applied under the various pH conditions. Figure 1 shows the relationship between the solution pH used for coprecipitation and the recovery of elements, which was de-

defined as the ratio of the increase in the amount of the spiked element detected by ICP-AES to the given amount of the spiked element. Pb and Bi were quantitatively recovered over a wide pH range. The recoveries of Al, Cr, Fe, Cd and Zn increased as the pH of the solution increased with quantitative recoveries were obtained at pH values above 5.5. On the other hand, the recoveries of Na and K were very low (2% and 3% at pH 6.0, respectively). Even for Mg and Ca, which are known to form insoluble phosphate salts, the recoveries were low (8% and 50% at pH 6.0, respectively) after the coprecipitation. These results indicate that yttrium phosphate is useful for the separation of Al, Cr, Fe, Zn, Cd, Pb and Bi from alkali and alkaline earth elements in crude-drug analysis.

The addition of more than 5 mg of yttrium did not influence the ICP-AES determination (data not shown). The quantitative recoveries of the elements were archived by adding more than 1 ml of 0.5 mol/l of phosphoric acid for Cd and 0.5 ml for the others. Based on these results, the coprecipitation was carried out using 5 mg of yttrium and 1 ml of 0.5 mol/l phosphoric acid solution at pH 6.0. The quantitative recovery of the precipitate was easily accomplished after centrifugation at 3000 rpm for at least 5 min. The separated precipitate was easily dissolved in 5 ml of 1.6 mol/l nitric acid.

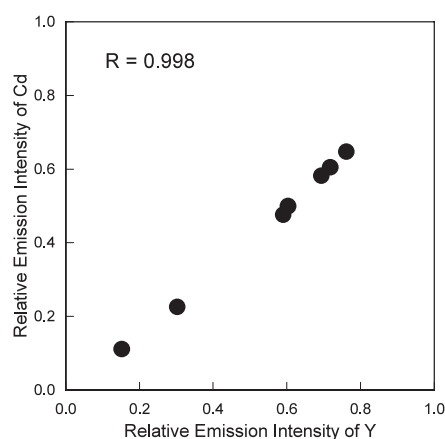
### Internal Standard Element

In this method, yttrium was added as a carrier element and also used as an internal standard element. The relationship between the emission intensities of yttrium and Cd is shown in Fig. 2. In this investigation, a portion of the precipitate was purposely discarded during the precipitation-separation process, and then the remaining precipitate was dissolved. The emission intensity of Cd was strongly

correlated with that of yttrium. Similar results were obtained for the other elements; the correlation coefficients ( $R$ ) for the straight lines were 0.996 for Al, 0.998 for Cr, 0.999 for Fe, 0.998 for Zn, 0.996 for Pb and 0.995 for Bi.

### Application to Analyses of Standard Reference Materials and Crude Drugs

To validate the proposed method, the elements in some standard reference materials (NIST SRM1515, apple leaves; SRM1547, peach leaves; and SRM1575a, pine needles) were determined. The values obtained by the proposed method were nearly equal to the certified values except for the values for Pb in SRM1515 and SRM1575a (Table 2). This discrepancy resulted because the Pb contents in these two SRMs were below the limit of quantification ( $10\sigma$ ,  $n = 10$ ) of the proposed method.



**Fig. 2.** Relationship between the Emission Intensities of Yttrium and Cd in the Solution After Coprecipitation  
Sample, solution (10 ml) after digestion of 1.0 g of Dioscorea Rhizoma; each element, 10  $\mu$ g; yttrium, 5 mg; 0.5 mol/l phosphoric acid, 1 ml; pH 6.

**Table 2.** Results for Standard Reference Materials

Element	NIST SRM1515 ( $n = 4$ )		NIST SRM1547 ( $n = 5$ )		NIST SRM1575a ( $n = 5$ )	
	Found <sup>a)</sup> (mg/kg)	Certified (mg/kg)	Found <sup>a)</sup> (mg/kg)	Certified (mg/kg)	Found <sup>a)</sup> (mg/kg)	Certified (mg/kg)
Al	279 $\pm$ 23	286	263 $\pm$ 21	249	568 $\pm$ 32	580
Cr	0.24 $\pm$ 0.008	0.3 <sup>b)</sup>	0.88 $\pm$ 0.090	1 <sup>b)</sup>	0.302 $\pm$ 0.006	0.3 – 0.5 <sup>b)</sup>
Fe	72 $\pm$ 0.442	80 <sup>b)</sup>	197 $\pm$ 3.6	220 <sup>b)</sup>	43 $\pm$ 0.3	46
Zn	12.3 $\pm$ 0.3	12.5	16.3 $\pm$ 0.38	17.9	36 $\pm$ 0.7	38
Cd	0.016 $\pm$ 0.004	0.014 <sup>b)</sup>	0.020 $\pm$ 0.005	0.03 <sup>b)</sup>	0.236 $\pm$ 0.009	0.233
Pb	0.483 $\pm$ 0.054 <sup>c)</sup>	0.470	0.958 $\pm$ 0.174	0.87	0.222 $\pm$ 0.028 <sup>c)</sup>	0.167
Bi	n.d.		n.d.		n.d.	

0.5 g of the sample was used for the determination. n.d.: Not detected.

a) Mean  $\pm$  S.D. b) The value is not certified; it is provided as additional information. c) The result was estimated by the extrapolation of the calibration curve.

**Table 3.** Results for Elements in Crude Drugs

Element	Concentration (mg/kg) <sup>a)</sup> (Recovery, %) <sup>b)</sup>						
	Puerariae Radix	Ephedrae Herba	Cinnamomi Cortex	Paeoniae Radix	Glycyrrhizae Radix	Zingiberis Rhizoma	Dioscoreae Rhizoma
Al	184 ± 19 (102)	168 ± 5 (93.2)	32.0 ± 4.4 (97.3)	203 ± 1 (91.4)	222 ± 10 (90.4)	71.7 ± 2.9 (107)	44.7 ± 1.1 (89.1)
Cr	1.18 ± 0.05 (91.9)	5.17 ± 0.25 (82.6)	4.64 ± 0.16 (90.7)	0.97 ± 0.06 (93.6)	1.20 ± 0.03 (107)	1.36 ± 0.02 (94.8)	0.47 ± 0.01 (95.2)
Fe	114 ± 2 (91.2)	125 ± 0.9 (76.1)	37.3 ± 0.7 (87.5)	123 ± 0.5 (85.9)	145 ± 1 (120)	66.3 ± 0.7 (93.3)	86.2 ± 0.9 (94.2)
Zn	9.51 ± 0.06 (85.6)	9.12 ± 0.10 (78.8)	4.78 ± 0.06 (85.0)	14.4 ± 0.1 (87.7)	11.9 ± 0.1 (90.4)	9.64 ± 0.18 (95.1)	5.41 ± 0.07 (95.7)
Cd	0.13 ± 0.004 (106)	0.03 ± 0.003 (78.3)	0.26 ± 0.003 (109)	0.16 ± 0.003 (115)	n.d. (102)	0.15 ± 0.002 (83.2)	0.01 ± 0.002 (89.3)
Pb	n.d. (90.0)	0.66 ± 0.05 (77.8)	n.d. (88.3)	0.94 ± 0.42 (93.4)	n.d. (103)	n.d. (91.8)	n.d. (98.4)
Bi	n.d. (98.4)	n.d. (85.3)	1.60 ± 0.07 (93.3)	n.d. (96.2)	n.d. (105)	0.36 ± 0.01 (97.2)	n.d. (98.2)

1.0 g of the sample was used for the determination. n.d.: Not detected.

a) Mean ± S.D. (n = 5). b) The solution was spiked with 50 µg of each element after microwave digestion.

The determination of elements in seven different kinds of crude drugs was then attempted. As a result, Al, Cr, Fe and Zn were determined in all of the crude drugs that were examined, as shown in Table 3. Among seven drug samples that were tested, trace levels of Cd were detected in six samples. A few samples also contained trace level of Pb and a few other samples contained trace levels of Bi.

To evaluate the effect of matrix elements in these drug samples on the determination of the seven elements being examined, recovery tests were performed. The results are shown in Table 3. Overall, 83.2–120% of the spiked elements were recovered from the crude drugs, except for Ephedrae Herba; the recoveries of Fe, Zn, Cd and Pb from Ephedrae Herba were all below 80%. Because the content of Ca in Ephedrae Herba was found to be more than  $2.38 \times 10^4$  mg/kg, which was much higher than that in the other crude drugs used for this investigation ( $5.50 \times 10^2$ – $1.13 \times 10^4$  mg/kg), the low recovery from Ephedrae Herba was most likely caused by the matrix effect of Ca on the determination.

In conclusion, the proposed method was successfully utilized to determine seven elements in seven kinds of crude drugs. This method is promising not only for the quality control of crude drugs, but also for providing drug traceability.

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