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Determination of microelements in Codonopsis Radix by inductively coupled plasma-mass spectrometry

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An inductively coupled plasma-mass spectrometry (ICP-MS) was to determine microelements such as Be, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Ba, Hg, Pb, Li, Mg, Ca, Ga, Rb, Sr, Mo, Ag, Sn and Cs with In as internal standard. The limits of detection (LOD) for elements mentioned earlier are between 0.003 and 2.478 ng/g, and the recoveries are between 92 and 112.20%. These results are in agreement with the reference values of standard reference materials in the poplar leaves. The Overall method is accurate, reliable and applicable in the determination of microelements in Codonopsis Radix, and the consideration of quantitatively analyzing elements in other Chinese traditional herbs.

Key words: *Codonopsis pilosula*, *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T.Shen, microelements, ICP-MS, quantitative analysis.

INTRODUCTION

Codonopsis Radix (“Dang shen” in Chinese), the root of three Campanulaceae Codonopsis plants: *Codonopsis pilosula* (Franch.) Nannf, *C. pilosula* Nannf. var. *modesta* (Nannf.) L.T.Shen, or *Codonopsis tangshen* Oliv recorded in the People’s Republic of China Pharmacopoeia (2010), is a famous traditional Chinese medicine (TCM), which has been widely used to treat some diseases and be accepted as edible tonic in China, Japan and Korea. The extract of Codonopsis Radix has been used as a traditional remedy for replenishing energy deficiency, strengthening the immune system, lowering blood pressure and improving appetite for a long history (Xu et al., 1996). There are more than 40 *Codonopsis* species around the world, in which as many as 39 species grow in Northwestern region of China. The quality of Codonopsis Radix in this district is much better than others, especially in Wen county of Gansu province, where this species is known as “Wen Dang” (*C. pilosula* Nannf. var. *modesta* (Nannf.) L.T.Shen) among the local people, and is generally accepted as one of the finest and exported to the rest of the world. In recent years, “Baitiao Dang” (*C. pilosula* (Franch.) Nannf), produced in Weiyuan county of Gansu province, has received attention owing to its large production.

Notably, microelements in Chinese herbs are closely related to TCM’s therapeutic effect. Ca can adjust the balance of excitation and inhibition process, and it owns the effect of reducing inflammation and swelling, anti-allergy and detoxification; Fe is an important component of hemoglobin and catalase, the deficit of which would lead to anemia; Zn is a constituent of more than 70 kinds of enzymes, and it is involved in synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids; Se can protect the cardiovascular system, prevent liver disease and cancer, and also eliminate Pb, Cd, Hg and other heavy metals from the human body. As a component of multi-enzyme system, Mn is involved in the formation of bone and connective tissue and bone

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growth. It is also indispensable in cholesterol synthesis and coagulation; Cu is involved in hematopoiesis, bone growth, and development of red and white blood cells; Co, Sr and Ni play an indispensible therapeutic role in gynecology, pediatrics and leukemia (Weaver and Heaney, 1999; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, 1997, 2001). Therefore, composition and content of microelements is of great significance in TCM. There is lack of literature on microelements in Codonopsis Radix. This study is intended to seek an ideal analysis method to determine trace elements in “Wen Dang” and “Baitiao Dang”. A rapid and accurate determination method to analyze micro-elements appears to be of great significance in the studies of TCM’s pharmacology and toxicology, and it also benefits authentication and quality control of traditional Chinese medicine (TCM).

Many analytical methods have been developed for determination of microelements in medicine herbs, such as atomic absorption spectrophotometry (AAS) and atomic fluorescence spectrometry (AFS). However, they can only determine one element at one time one. Inductively coupled plasma atomic emission spectrometry (ICP-AES) is able to achieve simultaneous determination of a variety of metal elements, but a relatively high limit of detection (LOD) limits its application (Schramel et al., 1995; Wang et al., 1999; Ong et al., 1999). Over the last decade, inductively coupled plasma-mass spectrometry (ICP-MS) has been rapidly developed and widely used as a fast, accurate, simultaneous determination of multi-element technique (Wang et al., 2006, 2009, 2011). The studies reported simultaneous determination of twenty-seven metallic microelements in Codonopsis Radix by ICP-MS coupled with microwave digestion. Furthermore, the microelement contents in Wen Dang were also compared with those in Baitiao Dang.

MATERIALS AND METHODS

Instruments

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Thermo X7 series); Microwave Digestion System (Milestone- E7hosd, Italy, CEM), including a Microwave oven, a PTEE-TEE high pressure digestion tank and fixing devices; Electronic balance (Mettler Toledo); Mill-Q water purification system.

Reagents and samples

Multi-element standard solution (Fluka) which contains twenty-seven elements to measure. Concentration: Be: 10 $\mu g \cdot g^{-1}$, B: 100 $\mu g \cdot g^{-1}$, Al: 40 $\mu g \cdot g^{-1}$, V: 40 $\mu g \cdot g^{-1}$, Cr: 20 $\mu g \cdot g^{-1}$, Mn: 10 $\mu g \cdot g^{-1}$, Fe: 100 $\mu g \cdot g^{-1}$, Co: 10 $\mu g \cdot g^{-1}$, Ni: 20 $\mu g \cdot g^{-1}$, Cu: 20 $\mu g \cdot g^{-1}$, Zn: 100 $\mu g \cdot g^{-1}$, As: 40 $\mu g.g^{-1}$, Se: 10 $\mu g \cdot g^{-1}$, Cd: 10 $\mu g \cdot g^{-1}$, Ba: 40 $\mu g \cdot g^{-1}$, Hg: 100 $\mu g \cdot g^{-1}$, Pb: 40 $\mu g \cdot g^{-1}$, Li: 10 $\mu g \cdot g^{-1}$, Mg: 100 $\mu g \cdot g^{-1}$, Ca: 100 $\mu g \cdot g^{-1}$, Ga: 10 $\mu g \cdot g^{-1}$, Rb: 20 $\mu g \cdot g^{-1}$, Sr: 40 $\mu g \cdot g^{-1}$, Mo: 10 $\mu g \cdot g^{-1}$, Ag: 100 $\mu g \cdot g^{-1}$, Sn: 10 $\mu g \cdot g^{-1}$, Cs: 10 $\mu g \cdot g^{-1}$. Internal standard: In standard solution (GBW (E) 080603, 1000 $\mu g \cdot g^{-1}$). Reference material: poplar leaf, GBW 07604 (GSW-3) 0513 (Institute of Giophysical and Geochemical Exploration, Ministry of Geology and Mineral, China). Nitric acid (MOS grade, Beijing Chemical Reagent Research Institute), hydrogen peroxide (G. R., Sinopharm Chemical Reagent Co. Ltd).

A total of twenty-seven samples (dried roots of *C. pilosula* (Franch.) Nannf. and *C. pilosula* Nannf. var. *modesta* (Nannf.) L.T.Shen) were collected from different regions in Gansu province, and identified by Professor Yuning Yan (Beijing University of Traditional Chinese Medicine). To ensure the authenticity of the raw materials, they were directly collected from Gansu Province (Table 1).

ICP-MS working parameters

With the internal standard, ICP-MS working parameters were automatically adjusted by the instrument to optimize sensitivity, background and stability of the method (Table 2). Isotopes of all metallic elements were also chosen for the same purpose. The concentration of Hg was measured in semi-quantitative mode, while the others were measured in all-quantitative mode.

Experimental

Preparation of standard solutions

For internal standard solution, In stock solution was diluted to 2 $\mu g \cdot kg^{-1}$ with ultrapure water, 5, 10, 25, 50, and 100 µl of multi-element standard solution (Fluka) was transferred into a 100 ml volumetric flask, then diluted to the required volume with ultrapure water, respectively. Mix evenly, and a series of different concentration standard solutions were prepared.

Preparation of samples

Reference material of 0.5 g poplar leaf was dissolved in 3 ml nitric acid and 2 ml hydrogen peroxide in a digestion tank. After keeping in the closed tank for 12 h, the solution was placed into the microwave oven and digested according to the set digestion procedure (Table 3). Then the solution were transferred to a 50 ml PET volumetric flask and diluted to a total of 25 ml volume with ultrapure water.

A sample of 0.5 g of powdered Codonopsis Radix was prepared in the same procedure as poplar leaf reference material. Meanwhile, control experiment was carried out in which only solvent was prepared in the same procedure as the aforementioned method.

RESULTS

Establishment of calibration curve

Ultrapure water and series of calibration standard solutions were injected into ICP-MS under the optimized experimental conditions. Standard curves of all elements were established. The blank solvent was injected consecutively for 6 times under the optimized experimental conditions, and the limits of detection (LOD) were calculated (Table 4).

Table 1. Sample information of different production place.

No.	Species	Place of origin	Growth state	Growth period (year)	Elevation (m)	Soil types	Collection time	Trade name
S1-A	*C. pilosula*	Qingyuan town, Weiyuan county	Wild type	-	2450	Black soil	2009.10	Baitiao Dang
S1-B	*C. pilosula*	Qingyuan town, Weiyuan county	Wild type	-	2450	Black soil	2009.10	Baitiao Dang
S1-C	*C. pilosula*	Qingyuan town, Weiyuan county	Wild type	-	2450	Black soil	2009.10	Baitiao Dang
S2-A	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2080	Black soil	2009.10	Baitiao Dang
S2-B	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2080	Black soil	2009.10	Baitiao Dang
S2-C	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2080	Black soil	2009.10	Baitiao Dang
S3-A	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2150	Black soil	2009.10	Baitiao Dang
S3-B	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2150	Black soil	2009.10	Baitiao Dang
S3-C	*C. pilosula*	Qingyuan town, Weiyuan county	Trained type	2	2150	Black soil	2009.10	Baitiao Dang
S4-A	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	2	2421	Lime soil	2009.10	Baitiao Dang
S4-B	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	2	2421	Lime soil	2009.10	Baitiao Dang
S4-C	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	2	2421	Lime soil	2009.10	Baitiao Dang
S5-A	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	3	2421	Lime soil	2009.10	Baitiao Dang
S5-B	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	3	2421	Lime soil	2009.10	Baitiao Dang
S5-C	*C. pilosula*	Lianfeng town, Weiyuan county	Trained type	3	2421	Lime soil	2009.10	Baitiao Dang
S6-A	*C. pilosula*	Xihua town, Huating county	Trained type	2	1350	Loess soil	2009.10	Baitiao Dang
S6-B	*C. pilosula*	Xihua town, Huating county	Trained type	2	1350	Loess soil	2009.10	Baitiao Dang
S6-C	*C. pilosula*	Xihua town, Huating county	Trained type	2	1350	Loess soil	2009.10	Baitiao Dang
S7-A	*C. pilosula*	Shanzhai town, Huating county	Trained type	2	1400	Loess soil	2009.10	Baitiao Dang
S7-B	*C. pilosula*	Shanzhai town, Huating county	Trained type	2	1400	Loess soil	2009.10	Baitiao Dang
S7-C	*C. pilosula*	Shanzhai town, Huating county	Trained type	2	1400	Loess soil	2009.10	Baitiao Dang
S8-A	*C. pilosula* var	Zhongzhai town, Wen county	Wild type	-	1450	Sandy soil	2009.10	Wen Dang
S8-B	*C. pilosula* var	Zhongzhai town, Wen county	Wild type	-	1450	Sandy soil	2009.10	Wen Dang
S8-C	*C. pilosula* var	Zhongzhai town, Wen county	Wild type	-	1450	Sandy soil	2009.10	Wen Dang
S9-A	*C. pilosula* var	Zhongzhai town, Wen county	Trained type	2	1380	Gray plastic soil	2009.10	Wen Dang
S9-B	*C. pilosula* var	Zhongzhai town, Wen county	Trained type	2	1380	Gray plastic soil	2009.10	Wen Dang
S9-C	*C. pilosula* var	Zhongzhai town, Wen county	Trained type	2	1380	Gray plastic soil	2009.10	Wen Dang

C. pilosula: *Codonopsis pilosula* (Franch.) Nannf.; *C. pilosula* var: *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T.Shen.

Table 2. Work parameter of ICP-MS.

Item	Forward power (w)	Sampling depth	Carrier ($L \cdot min^{-1}$)	Plasma ($L \cdot min^{-1}$)	Auxiliary gas ($L \cdot min^{-1}$)	Pump speed ($r \cdot min^{-1}$)	Nebulizer temp (°C)	Dwell time (ms)	Channels	Main runs	Sweeping times (ms)	Acquistion time (ms)
Parameter	1200	80	0.85	13.0	0.7	30	3	10	3	3	30	0.45

Table 3. Parameter of microwave digestion.

Steps	Rising-temp time (min)	Temperature (°C)	Hold time (min)	Power (w)
1	5	150	5	800
2	5	200	20	800

Table 4. Result of poplar leaves standard materiel and recovery test.

Inorganic element	LOD (ng/g^{-1})	Poplar leaves standard value ($\mu g/g^{-1}$)	Results ($\mu g/g^{-1}$)	Linear equations	Linear range (ng/g^{-1})	Correlation coefficient	Recovery (%)
Li^{7}	0.003	0.32±0.03	0.31	$Y=6.34\times10^{3}X+2.24\times10^{-1}$	0～10	0.9998	95.5
Be^{9}	0.066	0.021±0.005	0.018	$Y=2.12\times10^{3}X+2.22\times10^{-2}$	0～10	0.9999	92.0
B^{11}	0.171	53±5	50	$Y=2.98\times10^{3}X+3.10\times10^{-3}$	0～100	0.9997	96.2
Mg^{24}	2.563	474±17	498	$Y=7.08\times10^{3}X+2.34\times10^{-2}$	0～100	0.9996	98.2
Al^{27}	0.165	104±60	160	$Y=1.02\times10^{4}X+4.14\times10^{-2}$	0～40	0.9999	112.2
Ca^{40}	2.650	974±27	998	$Y=3.41\times10^{2}X+3.01\times10^{-2}$	0～100	0.9991	102.2
V^{51}	0.006	0.64±0.06	0.58	$Y=1.76\times10^{4}X+5.40\times10^{-2}$	0～40	0.9991	100.9
Cr^{52}	0.006	0.55±0.07	0.51	$Y=1.56\times10^{4}X+3.50\times10^{-1}$	0～20	0.9999	95.0
Mn^{55}	0.006	45±4	43	$Y=2.00\times10^{4}X+8.10\times10^{-2}$	0～10	0.9995	92.6
Fe^{56}	2.478	274±17	298	$Y=1.70\times10^{4}X+6.56\times10^{-3}$	0～100	0.9996	96.2
Co^{59}	0.003	0.42±0.03	0.41	$Y=1.78\times10^{4}X+2.22\times10^{-2}$	0～10	0.9996	92.3
Ni^{59}	0.006	1.9±0.3	1.60	$Y=4.18\times10^{3}X+6.67\times10^{-1}$	0～20	0.9993	99.2
Cu^{64}	0.009	9.3±1.0	9.90	$Y=4.90\times10^{3}X+2.92\times10^{-1}$	0～20	0.9999	96.9
Zn^{66}	0.078	37±3	35	$Y=3.09\times10^{3}X+2.46\times10^{-1}$	0～100	0.9997	96.2
Ga^{70}	0.003	13.4±2.2	13.5	$Y=1.54\times10^{4}X+2.34\times10^{-1}$	0～10	0.9991	93.5
As^{75}	0.042	0.37±0.09	0.36	$Y=2.54\times10^{3}X+1.08\times10^{-3}$	0～40	0.9999	100.8
Rb^{85}	0.006	3.4±3.2	3.5	$Y=2.21\times10^{4}X+7.30\times10^{-3}$	0～20	0.9998	96.5
Sr^{88}	0.003	14.4±4.1	15.4	$Y=1.66\times10^{4}X+2.06\times10^{-3}$	0～40	0.9997	93.5
Mo^{96}	0.006	0.34±1.8	0.35	$Y=4.99\times10^{3}X+7.39\times10^{-2}$	0～10	0.9994	99.8
Ag^{107}	0	0.32±0.07	0.29	$Y=8.38\times10^{3}X+2.07\times10^{-3}$	0～100	0.9968	96.8
Cd^{112}	0	0.32±0.07	0.29	$Y=3.37\times10^{3}X+1.45\times10^{-3}$	0～10	0.9995	93.1
Sn^{119}	0.003	0.04±0.02	0.05	$Y=9.30\times10^{3}X3+3.82\times10^{-3}$	0～10	0.9999	94.5
Cs^{133}	0.003	0.08±2.0	0.09	$Y=2.90\times10^{4}X+7.64\times10^{-2}$	0～10	0.9999	95.8
Ba^{137}	0.003	26±4	21	$Y=4.29\times10^{3}X+3.20\times10^{-3}$	0～40	0.9999	95.2
Hg^{200}	0.03	0.026±0.003	0.03	$Y=5.00\times10^{3}X+6.60\times10^{-2}$	0～100	0.9987	93.5
Tl^{204}	0.003	20.4±2.2	21.5	$Y=2.56\times10^{4}X+1.55\times10^{-2}$	0～100	0.9981	110.6
Pb^{207}	0.003	1.5±0.3	1.5	$Y=2.47\times10^{4}X+4.35\times10^{-3}$	0～40	0.9998	98.5

Determination of reference materials

In order to verify the reliability and accuracy of established method, reference material poplar leaf was injected into ICP-MS. The measured value was in accordance with the marked value (Table 4).

Determination of recovery

Per 0.4 g reference material, poplar leaf was dissolved in 5 ml nitric acid and kept in a closed digestion tank for 12 h, respectively. Each solution was mixed with 2 ml hydrogen peroxide and 2, 6, and 10 µl multi-element standard solutions, respectively. The digestion tank was placed into the microwave oven and digested according to the set digestion procedure. After the solution cooled to room temperature, it was transferred into a 25 ml volumetric flask and diluted to the marked volume using ultrapure water. An aliquot was injected into ICP-MS analysis. Recovery rates were calculated and the results are shown in Table 4.

Table 5. Result of twenty-seven microelement in Dangshen (μg.g^{-1} ppm).

Inorganic Element	S1	S2	S3	S4	S5	S6	S7	S8	S9
Li^{7}	0.65	0.34	0.36	0.41	0.40	0.53	0.52	0.41	0.20
Be^{9}	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
B^{11}	480.92	402.69	480.37	497.69	561.40	439.23	406.63	545.96	391.69
Mg^{24}	113.52	186.82	204.00	271.55	288.22	388.65	476.28	329.63	141.01
Al^{27}	850.31	707.07	919.15	959.85	920.63	792.52	736.07	1301.47	1063.71
Ca^{40}	0.98	1.04	0.90	0.96	0.99	1.16	1.32	1.00	0.52
V^{51}	1.07	1.17	0.78	0.87	1.11	1.15	1.18	1.30	0.64
Cr^{52}	7.96	10.65	12.17	12.81	15.01	11.72	14.33	12.65	6.71
Mn^{55}	149.09	211.81	238.52	317.77	307.52	408.05	467.24	302.35	115.36
Fe^{56}	0.09	0.11	0.12	0.15	0.16	0.17	0.19	0.15	0.06
Co^{59}	0.68	0.65	0.49	0.57	0.70	0.69	0.64	0.73	0.57
Ni^{59}	5.57	1.87	2.21	2.97	3.02	2.76	1.83	2.31	1.84
Cu^{64}	6.64	5.32	5.44	6.96	6.51	7.43	6.92	8.70	8.45
Zn^{66}	6.57	2.58	4.41	3.01	4.91	9.74	12.20	20.90	5.21
Ga^{70}	0.16	0.23	0.26	0.33	0.34	0.35	0.37	0.37	0.17
As^{75}	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01
Rb^{85}	4.46	3.28	3.67	4.12	3.94	2.69	1.63	2.73	2.76
Sr^{88}	22.85	10.95	16.69	15.80	17.50	10.89	10.29	20.93	22.23
Mo^{96}	0.18	0.22	0.20	0.20	0.20	0.18	0.22	0.40	0.20
Ag^{107}	0	0	0	0	0	0	0	0	0
Cd^{112}	0.01	0.01	0.01	0.01	0.01	0.04	0.02	0.09	0.13
Sn^{119}	0.07	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.03
Cs^{133}	0.04	0.06	0.07	0.09	0.08	0.10	0.11	0.09	0.04
Ba^{137}	96.10	43.29	65.21	49.13	71.80	134.32	178.95	304.85	79.88
Hg^{200}	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.00
Tl^{204}	0.04	0.03	0.03	0.03	0.03	0.03	0.01	0.02	0.02
Pb^{207}	0.41	0.34	0.34	0.35	0.36	0.40	0.40	0.40	0.27

Determination of microelements

Each sample solution prepared in determination of recovery was injected into ICP-MS analysis. Concentrations of twenty-seven metallic microelements in each Codonopsis Radix sample were determined (Table 5).

DISCUSSION

In this study, microwave was applied to digest samples shows major advantages: small sample volumes, short run time, low baseline value, and less element losses. Moreover, results in nitric acid-hydrogen peroxide system shows more reliable and accurate than those in single nitric acid digestion systems.

Using ICP-MS technology to determine the concentration of microelements makes up an easy, quick, sensitive, accurate and few-interference method. Metalic element In is uncommon in nature which would be seldom introduced from agents and utensils, so it is such a suitable internal standard that we adopted in the sample preparation procedure (Wang et al., 2006, 2009, 2011).

It can be seen from the results that Ca element has the highest content in Codonopsis Radix, followed Fe, Mg, Al, Ga, and Ba. Among them, Ca can keep the normal excitation of nerve; Fe is an indispensible metallic element for hematopoiesis; Mg is an activator of many enzymes in the process of cell metabolism, etc (Xu et al., 1996; Weaver and Heaney, 1999; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, 1997, 2001).

These functions of the aforementioned element may be consistent with the effect of Codonopsis Radix to tonify qi and invigorate spleen. In other words, pharmacological action of Codonopsis Radix may well be the result of common or synergistic effect of organic active ingredients and trace elements.

There are many factors that can affect the content of metallic microelements in TCMs, such as growth period, harvesting season, soil environment, water conditions, intensity and duration of sunshine, and chemical fertilizer used in the planting process (Xu et al., 1996). The microelements and their contents are both obviously

different in this analysis of Wen Dang and Baitiao Dang which further prove the earlier viewpoint. Baitiao Dang Li, Be, B, Mg, Ca, Cr, Mn, Fe, Ni, Rb, Sr, Sn, Cs, Tl, and Pb are significantly higher than Wen Dang; Baitiao Dang, in the S6 to S7 in Be, Mg, Ca, Mn, Fe and Cs are significantly higher than S1 to S5. Wen Dang Al, V, Co, Cu, Zn, Mo, Cd, and Ba are significantly higher than Baitiao Dang; in Wen Dang, wild type is significantly higher in content of microelements at trained type.

This report provides not only a scientific method for determination of microelements, but also a reference for the evaluation of quality and genuineness of Codonopsis Radix. Additionally, this method can be considerable be used to determine metallic elements in other TCMs.

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