

## Rapid Analysis of an Additive Medicinal Ingredient (Dried Thyroid) in Dietary Supplements

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**Dried thyroid added to dietary supplements was analyzed using liquid chromatography/mass spectrometry (LC/MS). However, rapid analysis of many dietary supplements on the market is difficult because the analysis uses expensive instruments (LC/MS) and requires a long time for pretreatment. The enzyme-linked immunosorbent assay (ELISA) is rapid, simple, and lowcost and thus suitable for processing many samples. In this study, we investigated rapid analysis of dried thyroid contained in dietary supplements with ELISA using commercial anti-thyroglobulin antibody. The analytical method using ELISA established in this study may be useful for the screening of dried thyroid added to dietary supplements.**

**Key words** — enzyme-linked immunosorbent assay, thyroglobulin, dietary supplement, medicinal ingredient, dried thyroid, rapid analysis

### INTRODUCTION

Impairment of health by dietary supplements combined with medicinal ingredients has been a social problem in recent years.<sup>1,2)</sup> To prevent impairment of health by medicinal ingredients contained in dietary supplements, it is necessary to analyze commercial dietary supplements as thoroughly as possible. Analytical methods are generally required to be highly sensitive, simple, rapid, and low in cost. Dried thyroid is one medicinal ingredient commonly added to dietary supplements.<sup>3,4)</sup> The current liquid chromatography/mass spectrometry (LC/MS) method<sup>5)</sup> for analyzing dried thyroid in dietary supplements is not adequate because it uses expen-

sive instruments, requires high technical skill, takes a long time to release a sufficient amount of thyroxine by enzymatic degradation of thyroglobulin, and involves complicated a solvent extraction.

Thyroglobulin is an iodine-containing macromolecular glycoprotein unique to the thyroid. Thyroglobulin binds to thyroxine and triiodothyronine and is present in colloids in the thyroid follicles, playing a major role in the storage of thyroid hormone and iodine. The enzyme-linked immunosorbent assay (ELISA) is an important substitutable screening method. Mikami *et al.* reported an analytical method for detecting dried thyroid-derived thyroxine using a commercial ELISA kit.<sup>6)</sup> Their method is low cost because no expensive instruments are used, but 28-hr enzyme treatment to release thyroxine from thyroglobulin is necessary. In this study, we investigated a rapid analytical method of the unique component of the thyroid thyroglobulin with ELISA using a commercial anti-thyroglobulin antibody.

### MATERIALS AND METHODS

**Reagents** — The following reagents were purchased: dried thyroid (Merck Hoei, Japan); thyroglobulin (Serva Electrophoresis, Germany); EIA plate (Greiner Bio-One, Japan); EZ-Link, Sulfo-NHS-LC-Biotin, and Immunopure NeutrAvidin, Horseradish Peroxidase conjugated (NAV-HRP) (Pierce, U.S.A.); polyclonal rabbit anti-human thyroglobulin (Tgab) (Dako, Japan); TMBZ (Dojindo Laboratories, Japan); albumin from bovine serum (BSA) (Sigma-Aldrich, U.S.A.); PD-10 (Amersham Bioscience, U.S.A.); and Blockace (liquid form) (Dainippon Pharmaceutical, Japan). For other reagents and dietary supplement samples, commercial products were used.

For the substrate solution, 100  $\mu$ l of TMBZ solution (10 mg/ml *N,N*,dimethylformamide) and

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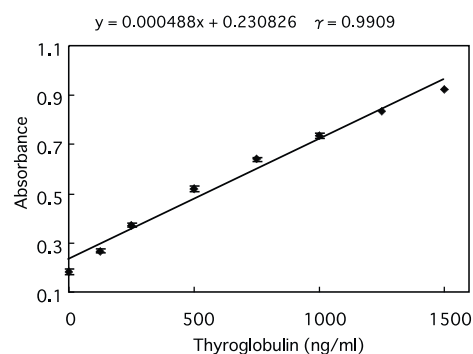
1.5  $\mu\text{l}$  of 30% hydrogen peroxide solution were mixed with 9.9 ml of acetate buffer immediately before use.

**Sample Solutions** — Capsules and tablets were powdered, and 1 g was weighed out. For liquid products, 1 ml was sampled. Ten and 9 ml of 0.5% BSA, pH 8.2, Tris-buffered saline 1 M containing 0.5% Tween 20 were added to the former and latter, respectively. The mixtures were shaken for 10 min and centrifuged at 3000 rpm for 10 min, and 2.5 ml of the supernatants were partially purified using PD-10, 0.5% BSA, pH 7.2, and phosphate-buffered saline (PBS) 10 mM containing 0.5% Tween 20 (PBST). The eluates were adjusted to 5 ml with 0.5% BSA/PBST and used as sample solutions.

**ELISA Analysis** — Tgab was added to 96-well microplates (10  $\mu\text{g}/\text{ml}$ , carbonate buffer, pH 9.6, 100  $\mu\text{l}/\text{well}$ ), and stored at 4°C overnight. After washing five times with PBST, Blockace five-fold diluted with PBS was added to the plates, and the plates were incubated for 1 hr for blocking. After washing five times with PBST, sample solutions of 100  $\mu\text{l}$  each were added to the wells and incubated for 1 hr. After washing five times with PBST, 100  $\mu\text{l}$  of biotin-labeled Tgab (0.27  $\mu\text{g}/\text{ml}$ , 0.5% BSA/PBST) was added to each well according to the manufacture's instructions for EZ-Link Sulfo-NHS-LC-Biotin, and the plates were incubated for 1 hr. After washing five times with PBST, 100  $\mu\text{l}$  of NAV-HRP (0.2  $\mu\text{g}/\text{ml}$ , 0.5% BSA/PBST) was added, and the plates were incubated for 30 min. After washing five times with PBST, 100  $\mu\text{l}$  of substrate solution was added, and the plates were incubated for 30 min. The reaction was stopped by adding 100  $\mu\text{l}$  of phosphoric acid 1 M, and the absorbance at 450 nm was measured.

## RESULTS AND DISCUSSION

Figure 1 shows an example of the standard curve of thyroglobulin within a range from 0 to 1500 ng/ml. The standard curve showed good linearity. Since 0.5% BSA/PBST was used for partial purification of sample solutions using PD-10, 0.5% BSA/PBST was used for the diluent. Table 1 shows the results of recovery of eight dietary supplements (A–H), to which thyroglobulin 500 and 1500 ng/ml was added. The recovery rates were satisfactory (91–111%). Many dietary supplements contain various additives (vitamin C, vitamin B group, *etc.*). Since thyroglobulin has a high molecular weight (about 670000),



**Fig. 1.** Typical Standard Curve of Thyroglobulin

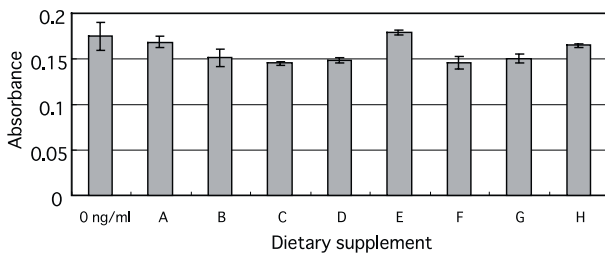
**Table 1.** Thyroglobulin Recovery Rates in Eight Dietary Supplements (%) ( $n = 3$ )

supplement	500 ng/ml	1500 ng/ml
A	108.16	110.82
B	94.61	99.15
C	105.88	110.35
D	99.63	102.84
E	99.77	99.00
F	91.27	96.10
G	102.21	103.71
H	100.96	105.20

**Table 2.** Additive Dried Thyroid Recovery Rates in Eight Dietary Supplements ( $n = 3$ )

A	96.27
B	59.53
C	85.93
D	60.66
E	88.18
F	44.59
G	114.16
H	112.80

sample solutions were subjected to separation from low molecular-weight substances and buffer exchange using PD-10, which separates high molecular-weight substrates ( $\text{MW} > 5000$ ) from low molecular-weight ones ( $\text{MW} < 1000$ ). To the eight dietary supplements, 10 mg of dried thyroid was added, and the test was performed as described above. Compared with the results with 10 mg of dried thyroid alone (without dietary supplements), the recovery rate was 45–110% (Table 2). To confirm the specificity of this test system, dietary supplements without dried thyroid were examined. The concentration in the dietary supplements without



**Fig. 2.** Measured Values of Background (0 ng/ml) and Eight Dietary Supplements without Dried Thyroid

dried thyroid was 0 ng/ml (background), showing no significant difference in the absorbance (Fig. 2).

The recovery rate was low in some samples, but thyroglobulin was detectable. Establishment of cut-off values is a major problem in ELISA. The cutoff value was established based on the detection limit according to the 14th revision of the *Japanese Pharmacopoeia*. The cutoff value was calculated as follows and calculated in each analysis. The slope near the cutoff value within the range of 0–500 ng/ml was used for the calculation.

$$\text{Cutoff value} = 3.3\sigma/\text{slope}.$$

Where  $\sigma$  is the standard deviation of 0 ng/ml (standard curve), and slope is the slope of the standard curve. When the measured value exceeded this detection limit, the sample was judged to contain dried thyroid.

The cutoff value calculated using the equation above was 21–78 ng/ml. Even when the recovery rate was the lowest, 335 ng/ml was detected in 10 mg of dried thyroid, which was sufficiently higher than the cutoff value, suggesting that this measurement system is capable of detecting 10 mg of dried thyroid. Based on the administration methods of pharmaceutical dried thyroid preparations and previous examples of the detection of dried thyroid in dietary supplements, 10 mg or more of dried thyroid may be contained in dietary supplements. The previous

method required more than 28 hr for the preparation of samples alone, but the method established in this study not only simultaneously processes many samples but also completes analysis within a few hours. The measurement system using ELISA is rapid, simple, and low cost and may be useful for the screening of dried thyroid contained in dietary supplements.

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## REFERENCES

- 1) Moriyasu, T., Shigeoka, S., Kishimoto, K., Ishikawa, F., Nakajima, J., Kamimura, H. and Yasuda, I. (2001) Identification system for sildenafil in health foods. *Yakugaku Zasshi*, **121**, 765–769.
- 2) Kumasaka, K., Kojima, T., Doi, K. and Satoh, S. (2003) Analysis of the oral hypoglycemic agent, glibenclamide, in a health food. *Yakugaku Zasshi*, **123**, 1049–1054.
- 3) Miyazawa, N., Nagahama, Y., Tadaki, S., Horie, M., Nozaka, T. and Hirose, Y. (2003) Analysis of fenfluramine, N-nitrosfenfluramine and thyroid hormone in Chinese dietary supplement. *Annual Report of Saitama Institute of Public Health*, **37**, 81–84.
- 4) Kumasaka, K., Miyazawa, M., Kojima, T., Doi, K. and Satoh, S. (2003) Summary of added medicines in health foods advertising weight reduction. *Annual Report of Kanagawa Prefectural Institute of Public Health*, **33**, 114–116.
- 5) Hamada, H. and Kosaka, T. (2000) Determination of thyroid hormones in health foods. *Annual Report of the Miyazaki Prefectural Institute for Public Health and Environment*, **12**, 83–88.
- 6) Mikami, E., Ohno, T., Matsumoto, H. and Sekita, S. (2003) Detection of thyroxine in dietary supplements using an enzyme-linked immunosorbent assay. *J. Health. Sci.*, **49**, 547–550.