# Stability of high-dose methylcobalamin injection

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease. It is treated with an active form of vitamin B12, methylcobalamin (mecobalamin). Recently, it has been reported that high-dose treatment with methylcobalamin may delay the progression of ALS. We have manufactured high-dose injections of methylcobalamin as a hospital formulation; however, the stability of the injection was unclear. Here we examined the stability of the injection using high performance liquid chromatography (HPLC). Methylcobalamin solution (12.5mg/ml, diluted with saline) was sealed in a brown glass ampoule under dark conditions (1-2 lux). The ampoules were sterilized by autoclaving (115°C, 30 minutes), and then protected from light with aluminum foil. After the ampoules had been kept under cool conditions (4°C) for 3 months, the concentration of methylcobalamin was found to be 96.4 ± 2.4% (mean ± S.E.) of the control value. After 6 months, methylcobalamin had decreased to 91.6 ± 1.4% (mean ± S.E.). In contrast, methylcobalamin quickly degenerated to 49.9 ± 8.2% (mean ± S.E.) of the control after only four hours' exposure to bright light (1,000 lux). In conclusion, the results suggest that injections of high-dose methylcobalamin can endure an autoclave, but methylcobalamin rapidly deteriorates with exposure to light. Thus, high-dose methylcobalamin injections should be prepared under dark conditions to prevent light-dependent degeneration.

**Keywords:** amyotrophic lateral sclerosis, high-dose methylcobalamin, stability

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative motor neurone disease (Catherine, 2004). There is currently limited therapy for ALS; riluzole is the only available approved medication that prolongs survival of patients with ALS (Lacomblez *et al*. 1996; Cheung *et al*. 2006). Recently, an active form of the vitamin $B_{12}$ analogue, methylcobalamin (mecobalamin), was introduced as a useful drug to improve the condition of ALS patients. Methylcobalamin is well absorbed, facilitating nucleic acid and protein biosynthesis, and axon repair. It has been suggested that high-dose methylcobalamin might be of clinical use for patients with peripheral neuropathies (Watanabe, 1994). Kaji *et al*. (1998) have also reported that high-dose treatment with methylcobalamin delays the progression of ALS according to the decreased index of spinal motor neurones, and compound muscle action potential. High-dose methylcobalamin injection is not currently supplied by pharmaceutical companies, and so was manufactured as an original formulation in our hospital, although its stability had not been clearly established. In this report, we analyzed the stability of high-dose methylcobalamin injection in respect of temperature and light.

## MATERIALS AND METHODS

### 1. Chemicals

Methylcobalamin was purchased from Sigma (St. Louis, MO, USA). Physiological saline was purchased from Fuso Pharmaceutical Industries, Ltd. (Tokyo, Japan) and brown glass ampoules were purchased from Mita Rika Kogyo Co., Ltd. (Tokyo, Japan). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) of analytical grade.

### 2. HPLC analysis

The HPLC system consisted of an LC-10AS HPLC pump, an SPD-6A UV detector, and a C-RSA recorder (Shimazu Corporation, Kyoto, Japan). Column temperature was maintained at 40°C in a CTO-6A column oven (Shimazu Corporation, Kyoto, Japan). Samples (20 $\mu$l) were injected on a reverse-phase column (Mightysil RP-18 GP250-4.6, 250×4.6 mm, i.d. 5 $\mu$m, Kanto Chemical. Co. Ltd, Japan). Quantitative analysis was performed according to the Japanese Pharmacopoeia 14th edition (website: http://jpdb.nihs.go.jp/jp14e/14data/Part-I/Mecobalamin.pdf). Operational conditions were as follows: detector, ultraviolet absorption photometer wavelength was 266 nm; mobile phases, 2:8 (v/v) comprising the ratio of acetonitrile and 0.02 mol/L $Na_2HPO_4$-tartaric acid (pH 3.5). The mobile phase finally contained 0.02mol/L of sodium 1-heptane sulfonate. The flow rate was 1 ml/minute and the internal standard solution was 5mg of riboflavin dissolved in 25 ml of methanol and added to 75 ml water. Then 10ml of the riboflavin solution was diluted with 40ml of 0.02mol/L $Na_2HPO_4$-tartaric acid (pH 3.5) solution. Methycobalamin solution for the standard was made just before quantitative analysis.

### 3. Preparation of the high-dose methycobalamin injection

Methylcobalamin was dissolved with saline at a concentration of 12.5mg/ml for 1 hour at room temperature under dark conditions. Methylcobalamin solution was aliquoted into brown glass ampoules and filtrated through a hydrophilicity filter (pore size: 0.2$\mu$m), also under dark conditions

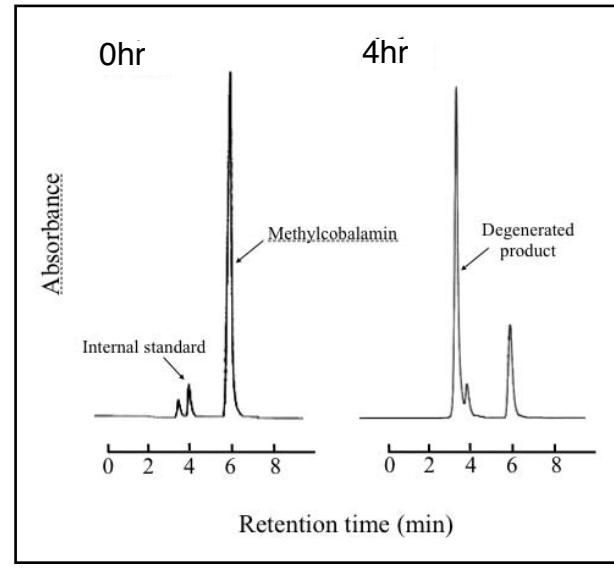


**Figure 1.** A typical chromatogram of high-dose methylcobalamin injection just after dissolution (0 hr) and after light exposure for 4 hours (1,000 lux, 4 hr). Each peak retention time was as follows: methylcobalamin, 5.9 minutes; internal standard, 4 minutes; degenerated product of methylcobalamin, 3.6 minutes.

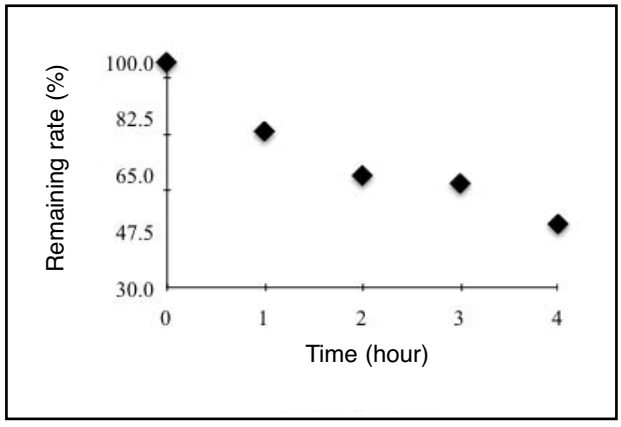


**Figure 2.** The remaining ratio of methylcobalamin after light (1,000 lux) exposure. Data are shown as the mean ± S.E. (n=4). The rates of remaining methylcobalamin after light exposure for 1, 2, 3 and 4 hours were decreased to 78.6 ± 4.7%, 64.9 ± 4.3%, 62.5 ± 2.5% and 49.9 ± 8.2% (mean ± S.E.) of control values, respectively.

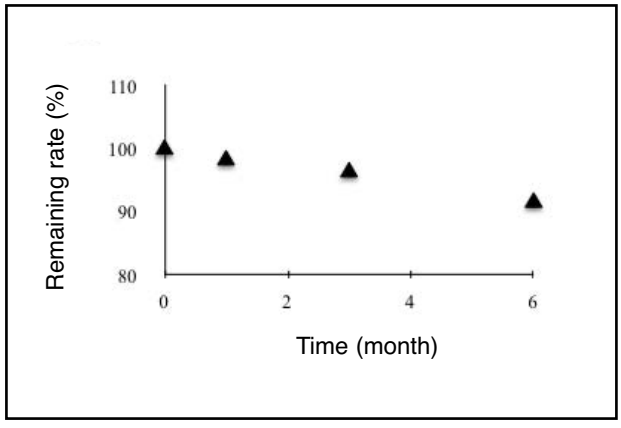


**Figure 3.** The remaining ratio of methylcobalamin injections in a refrigerator with shading. Data was shown as the mean ± S.E. (n=4). The remaining rates of methylcobalamin after 1, 3 and 6 months was 98.3 ± 3.3%, 96.4 ± 2.4% and 91.6 ± 1.4% (mean ± S.E.) of control values, respectively.

(1-2 lux). The ampoules were sterilized in an autoclave (115°C, 30 minutes) and then entirely shaded with aluminum foil. The ampoules were kept in cool conditions (4°C) until analysed.

## RESULTS

### 1. Quantitative analysis of methylcobalamin

Methylcobalamin and the internal standard were clearly separated by the HPLC system. The retention times of methylcobalamin and the internal standard were 4 minutes and 5.9 minutes, respectively. Degenerated product from methylcobalamin was detected as a peak with a retention time of 3.6 minutes (**Figure 1**).

### 2. Stability analysis of methylcobalamin solution by exposure to light

Methylcobalamin solution was exposed to light (fluorescent light, 1,000 lux) for 1, 2, 3 and 4 hours. Methylcobalamin was found to be degenerated by light; the methylcobalamin solution being clear red just after dissolving, but after exposure to light it changed to dark red. The methylcobalamin remaining after light exposure for 1, 2, 3 and 4 hours was decreased to 78.6 ± 4.7%, 64.9 ± 4.3%, 62.5 ± 2.5% and 49.9 ± 8.2% (mean ± S.E.) of the initial concentration, respectively (**Figure 2**). Correspondingly, degenerated products from the methylcobalamin gradually increased after light exposure. **Figure 1** shows a chromatogram of methylcobalamin solution just after dissolution in dark conditions (0 hr) and following exposure to light (1,000 lux) for 4 hours.

### 3. Stability analysis of the high-dose methylcobalamin injection

Methylcobalamin ampoules were sterilized by autoclaving at 115°C for 30 minutes. The ampoules were protected from light with aluminum foil and preserved in cool conditions (4°C). Methylcobalamin was found to breakdown only slowly, the remaining methylcobalamin after 1, 3 and 6 months being 98.3 ± 3.3%, 96.4 ± 2.4% and 91.6 ± 1.4% (mean ± S.E.) of the initial value, respectively (**Figure 3**).

## DISCUSSION

We studied the stability of high-dose methylcobalamin injection as a hospital formulation using HPLC analysis. The peaks of methylcobalamin, internal standard (riboflavin) and degenerated product were clearly separated by HPLC as illustrated in **Figure 1**. Methylcobalamin was rapidly converted to hydroxocobalamin in the presence of light, hydroxocobalamin being a major breakdown

product of methylcobalamin (http://www2.eisai.co.jp/di2/IF/MBL_A_IF/index.html: drug information of a commercial pharmaceutical methylcobalamin injection, Methycobal®).

The stability of methylcobalamin injection following autoclaving was found to be 98.3 ± 3.3% of the initial control value one month later. This result suggests that methylcobalamin is relatively heat stable, however, methylcobalamin does gradually degenerate with time. Commercially available methylcobalamin (Methycobal®) ampoules contain 500$\mu$g/ml/ampoule in 5% mannitol solution, and its quality as an injectable medicine is guaranteed for three years at room temperature. In contrast, the high-dose methylcobalamin injection contains 12,500 $\mu$g methylcobalamin/ml in 0.9% NaCl solution. The high concentration solution may accelerate the methylcobalamin denaturation. The Japanese Pharmacopoeia 14th edition defines that the amount of content in pharmaceutical prescriptions has to be between 95-110% of the pure substance described on the label (http://jpdb.nihs.go.jp/jp14e/). Thus, the high-dose methylcobalamin injection manufactured locally in our hospital may be stored for up to three months, but not longer.

In conclusion, this study has demonstrated that high-dose methylcobalamin is easily broken down by light, but is relatively stable to autoclaving. Thus, to retain their stability and quality for up to three months, it is necessary to both manufacture and store these high-dose methylcobalamin ampoules away from light and under cool conditions.

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