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Letter to the Editor

Comments on ''High-performance liquid chromatographic determination of methyl 6-mercaptopurine nucleotides in red blood cells''. Reply to R. Boulieu and T. Dervieux

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This letter is written in response to that submitted by Boulieu and Dervieux [1] concerning our paper entitled ''Reversed-phase high-performance liquid chromatographic assay method for quantitating 6-mercaptopurine and its methylated and non-methylated metabolites in a single sample'' [2].

The preparation of samples by us is not exactly the same as that by Boulieu and Dervieux [3,4]. We diluted erythrocytes with two times volumes of 0.02M KH_2PO_4 to obtain a 1 ml aliquot of red blood cells [2]. This procedure might influence the pH of the samples in subsequent procedure and the conversion rate of 6-methylmercaptopurine (6MMP) into its derivative. Several chromatographic conditions are also different as shown in Table 1. In our analytical condition, 6MMP is well detectable even after heating procedure. We agree, as mentioned in our paper [2], that 6MMP is decomposed by heat, but, we do not agree that 6MMP is completely converted into its derivative in our analytical condition. We observed a linear relationship between the concentration of heated (x) and non-heated (y) 6MMP; $y=8.949x$-313.269, $r^2=0.997$, $p=0.016$ (Fig. 1). Thus, it is possible to estimate 6MMP

Table 1
The chromatographic conditions by Mawatari et al. and by Boulieu and Dervieux

	Mawatari et al.	Boulieu and Dervieux
Column temperature	40°C	ambient
Mobile phase	0.02M phosphate buffer (pH 6.4), 0.1% 1-heptanesulphonic acid sodium salt	0.02M potassium phosphate (pH 3.5)
Flow-rate (ml/mm)	2.5	1.2
Concentration of methanol	varied from 0% at 4 min to 14% at 15 min	varied from 0 to 20% over a period of 12 min
Detection wavelength for 6MMP	289 nm	304 nm

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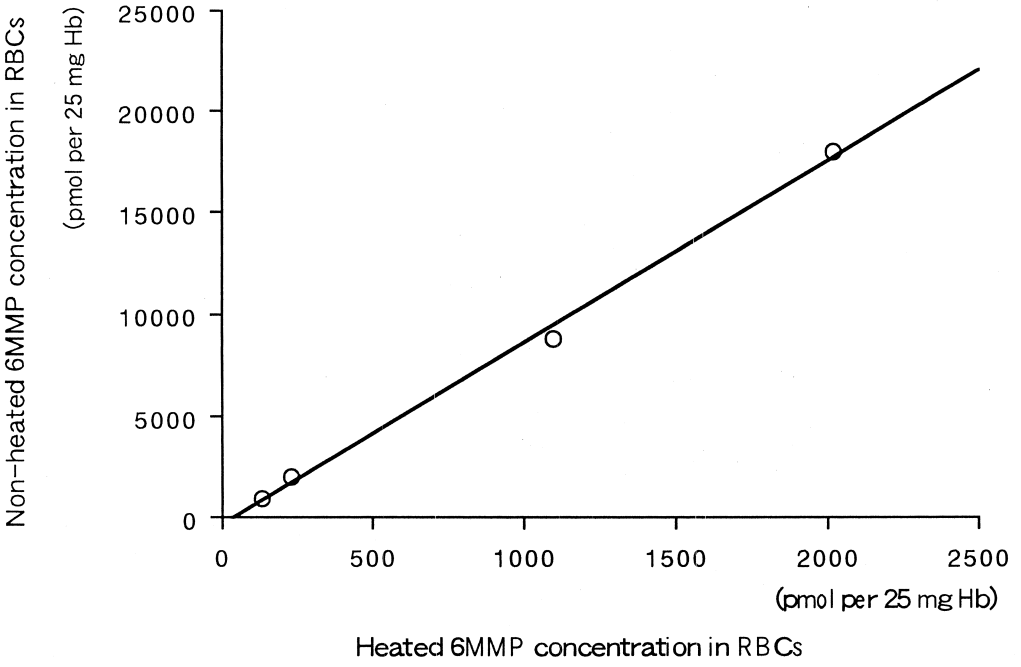

Fig. 1. Correlation between the concentration of heated (x) and non-heated (y) 6MMP; $y=8.949x$-313.269, $r^2=0.997$, $p=0.016$.

concentration in red blood cells. In conclusion, our HPLC assay method is appropriate for quantitating 6MMP in red blood cells.

We thank Boulieu and Dervieux for the mass spectrometric identification of 6MMP derivative, 4-amino-5-(methylthio) carboxy imidazole. In the chromatogram of our assay, the derivative is eluted after the large peak of dithiothreitol. We would like to estimate the relationship of 6MMP and its derivative.

References

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