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Letter to the Editor

High-performance liquid chromotographic determination of methyl 6-mercaptopurine nucleotides (Me6-MPN) in red blood cells: analysis of Me6-MPN per se or Me6-MPN derivative?

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In a recent paper published in this journal Mawatari et al. [1] report a HPLC method for the assay of 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and methyl 6-mercaptopurine (Me6-MP) in a single sample. With regard to some comments made by the authors on the stability of Me6-MP, we would like to discuss some important points concerning the analysis of Me6-MP nucleotides in biological samples.

As previously reported [2,3] Me6-MP is converted into a derivative during the acid hydrolysis step required to hydrolyse the thionucleotides in their own bases. The derivative formed can be analysed by HPLC after liquid-liquid extraction [2] with recovery lower than 40%. An other approach was a perchloric acid deproteinization with dithiothreitol followed by the heating step of the acid extract. With this sample treatment procedure, recovery of 84% for Me6-MPN derivative was found [3].

In regard with the instability of Me6-MP per se under acid conditions [3] and the sample treatment conditions used by Matawatari et al. [1] the identification of the compound eluted at the retention time of Me6-MP would be reconsidered. At the pH value required for the hydrolysis of thiopurine nucleotides into their free bases, Me6-MPN is converted into a derivative. The formation of Me6-MP derivative is strongly influenced by the pH of the acid extract.

In the sample treatment conditions reported by Mawatari et al. [1] that are the same than those previously reported [3], ie 100 μl of perchloric acid (70%) added to a 1 ml aliquot of red blood cells, the degree of conversion of Me6-MP to its derivative was about 100% [3]. Thus, considering the instability of the compound of interest, data on analytical recovery of Me6-MP would be also of interest.

To conclude, the determination of Me6-MP per se appeared technically difficult due to its instability in acidic conditions. The analysis of its derivative, 4-amino-5-(methylthio) carboxy imidazole, we have recently identified using liquid chromatography-mass spectrometry and spectrometric methods [4] represents an interesting approach for the accurate determination of methyl 6-mercaptopurine nucleotides in red blood cells from patients under thiopurine therapy.

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