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Poster

## [P25-6] P25-6: Immunosuppressive drugs (1): LC-MS/MS assay

Chair: Tsutomu Nakamura, Japan

Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

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(Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall )

## [P25-6-6] Simultaneous determination of 6-thioguanine nucleotides and 6-methylmercaptopurine in the red blood cells of patients with Crohn's disease by HPLC-DAD

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Keywords: azathioprine, 6-thioguanine nucleotides, 6-methylmercaptopurine, Crohn's disease, HPLC-DAD

### Background

In this study, a precise HPLC-DAD method has been developed and validated for the simultaneous determination of 6-thioguanine nucleotides (6-tgn) and 6-methylmercaptopurine (6-mmp) in patients with Crohn's disease (CD).

### Methods

The red blood cells (RBCs) were deproteinated with 100  $\mu$ L 70% perchloric acid, and then heated under acidic conditions. The analytes were separated on a reversed phase C18 column (TSKgel ODS-C18, 4.6  $\times$  250mm, 5  $\mu$ m) with a mobile phase of methanol -20mmol·L<sup>-1</sup> phosphate buffer (15:85, v/v) using isocratic elution. The flow rate was 0.9 min·L<sup>-1</sup> and the detection wavelengths were 340 nm and 310 nm, respectively. Red blood cell samples from 5 patients with CD were collected and quantified.

### Results

The calibration curves were linear over the range of 12-2400 pmol/(8 $\times$ 10<sup>8</sup>) RBC and 0.5 -200 nmol/(8 $\times$ 10<sup>8</sup>) RBC for 6-tgns and 6-mmp, respectively. From all 5 patients samples, the average concentration of 6-tgns and 6-mmp were (331.2 $\pm$ 185.0) pmol/(8 $\times$ 10<sup>8</sup>) RBC and (3.6 $\pm$ 1.9) nmol/(8 $\times$ 10<sup>8</sup>) RBC, respectively.

### Conclusions

The method can be applied to monitor 6-tgns and 6-mmp concentrations in red blood cells from patients under azathioprine therapy.