Abstract

Chronic inflammatory bowel disease, defined as recurrent inflammation of bowel, has spread to a global disease with increasing incidence in industrialized countries over the last century. The current goal of the treatment is the remission of the disease and it can take several stages depending on the complexity of the disease.

Azathioprine, a purine antimetabolite is an immunosuppressive drug used in the treatment of chronic inflammatory bowel disease as a standalone drug or in combination with biological agents from the group of tumor necrosis factor alpha inhibitors. It is a prodrug that undergoes metabolic conversion into two groups of active metabolites: 6-thioguanine nucleotides, carriers of therapeutic effect and 6-methylmercaptopurine nucleotides, which are hepatotoxic and their therapeutic effect has not been confirmed yet. For the optimal treatment tailored therapy dosing is recommended based on the concentration of active metabolites and the genotype of the metabolic enzyme thiopurine *S*-methyltransferase.

The analytics of both groups of metabolites is well developed and the established biological matrices are whole blood and red blood cells (RBC). Both matrices require invasive blood collection and demanding sample preparation due to the nature of the biological material. The stability of analytes is generally limited. An alternative to the established biological matrices are dried blood spots (DBS), where a drop of blood is taken from a finger or a heel and transferred to a DBS paper. It is also possible to use venous blood, which is applied to a DBS paper with a suitable pipette.

In the research work we developed and validated the liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of azathioprine metabolites by the dried blood spot technique. The method involves extraction from 30 µl of dried blood spot, hydrolysis, and quantification of 6-thioguanine and 6-methylmercaptopurine by LC-MS/MS method. The method is selective, linear, accurate, and precise in the range of 50 - 5300 pmol/8×10^8 RBC for 6-thioguanine and 260 - 5300 pmol/8×10^8 RBC for 6-methylmercaptopurine. The dilution integrity test demonstrated that the upper limit of concentration can be increased up to 8000 pmol/8×10^8 RBC for both analytes, thus covering both the therapeutic range for 6-thioguanine and the lower limit of the toxic range for 6-methylmercaptopurine. The absolute and relative matrix effects are low and the recovery is ≥ 80 %. We confirmed that different hematocrit values, different application volume, and sampling from different parts of the blood spot does not affect the accuracy and precision of the method. Both analytes are stable in dried blood spots for at least one month in the temperature range from -80 to 40 °C. Clinical validation confirmed that DBS method and routine clinical method with hemolysate samples give comparable results and enable similar clinical decisions.

The new developed method is simple and due to the many advantages of the dried blood spot technique it represents an alternative to established methods for the therapeutic drug monitoring of azathioprine metabolites.