Abstract

Nowadays, high-performance liquid chromatography is widely used analytical method for separation and quantification of drugs in biological material due to its high sensitivity and selectivity. HPLC is suitable for quantitative as well as qualitative analysis.

2-benzoylpyridine-4-ethyl-3-thiosemicarbazone (Bp4eT) is a potential drug from the group of thiosemicarbazones, which are currently intensively studied and developed as anticancer agents. Their specific mechanism of action, based on chelation of iron, might overcome resistance to standard chemotherapy.

The aim of this study was to develop and assess essential validation parameters of an HPLC-MS/MS method for determination of Bp4eT and its main phase I metabolites in rat plasma and to utilise the method for the analysis of samples from a pilot pharmacokinetic study in rats.

The separation of Bp4eT and its metabolites was achieved on chromatographic column Discovery HSC18 (75 x 4.6 mm, 3 µm) protected by the same type of guard column using the mobile phase consisting of ammonium formate and acetonitrile in ratio 40:60 (*v/v*). The isocratic elution and the flow rate of 0.4 ml/min were utilised. Mass spectrometer was chosen as a detector and the quantification was performed in selected reaction monitoring mode. Plasma samples were treated using the solid phase extraction. The method was successfully validated and utilised for the analysis of samples from *in vivo* study in rats, where Bp4eT was intravenously administered to four male rats and blood was collected in predefined time intervals.

The main pharmacokinetics parameters for Bp4eT were calculated and basic pharmacokinetic profiles of Bp4eT and M2 metabolite in rat plasma were determined.

Further effort will be focused on analytical evaluation of thiosemicarbazones from the group of di-2-pyridylketone-3-thiosemicarbazone which express higher efficacy, more appropriate pharmacokinetic properties and lower rate of side effects.