Abstract

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Title: Analytical and bioanalytical assessment of novel anticancer drugs

Pharmaceutical analysis plays an indispensable role in the development of novel safer, cheaper and more effective drugs and chaperones them from their first synthesis throughout their existence. To date, the method most commonly used in pharmaceutical analysis is liquid chromatography (LC) with various detection modalities. Separation via LC enables analysis of very complex mixtures such as biological samples or samples from stability studies. The soaring interest in LC hyphenated with mass spectrometry (MS) in pharmaceutical analysis is partly due to its increasing affordability and mainly due to its ability to separate the molecules of the analyte according to their mass-to-charge ratio (*m/z*). The additional dimension of separation grants it a superior selectivity. In addition, MS provides priceless information on the structure of the analyte – be it the *m/z* or fragmentation spectra.

In its theoretical part, this dissertation deals with basic description of liquid chromatography, mass spectrometry, validation of analytical and bioanalytical methods, stability assessment, pharmacokintetics and the analytes of interest. The second part, the commentary on the published work, consists of two units: **1) bioanalytical assessment** of pharmacokinetics of thiosemicarbazones – novel anticancer agents, freshly under clinical trials and **2) analytical assessment** of stability of carfilzomib – a new proteasome-inhibiting anticancer drug.

In the first unit, I discuss our bioanalytical approaches for assessment of metabolism and pharmacokinetics of three generations of promising anticancer drugs both *in vitro* and *in vivo*, especially aiming at their development and application. Using the developed method we found that: **1)** the studied – structurally close – thiosemicarbazones differ in pharmacokinetics and metabolism both qualitatively and quantitatively; **2)** the bulky substitution on the terminal

nitrogen protects the thiosemicarbazones from metabolism and the consequential loss of pharmacological effect; **3)** the toxic concentrations of the metabolites are rather unlikely to occur *in vivo* under standard dosing and **4)** the different pharmacokinetic and toxicity profiles of these three generations of anticancer drugs are not linked.

In the second unit of the experimental part, I comment on the first published stability indicating method for carfilzomib – a new anticancer drug – that we applied to stability assessment of this drug and to identification of possible degradation products. This method might be further used in development of potential future generic, in potential experimental development of alternative formulations or as a starting point for new methods for determination of carfilzomib.