Abstract

Determination of the total content of drug-related chlorine and chlorine speciation in human blood plasma using high performance liquid chromatography – tandem ICP-mass spectrometry (HPLC-ICP-MS/MS)

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In a first phase of this work, a fast, accurate and precise method for the separation and determination of the total contents of drug-related Cl and Br in human blood plasma, based on high performance liquid chromatography - inductively coupled plasma - tandem mass spectrometry (HPLC-ICP-MS/MS), has been developed[1]. The novel approach developed has been proved to be a suitable alternative to the presently used standard methodology (*i.e.* based on a radiolabelled version of the drug molecule and radiodetection), while eliminating the disadvantages of the latter. Interference-free determination of ^{35}Cl has been accomplished via ICP-MS/MS using H_2 as reaction gas and monitoring the $^{35}ClH_2^+$ reaction product at mass-to-charge ratio of 37. Br could be measured "on mass" at a mass-to-charge of 79. HPLC has been relied on for the separation of the drug-related entities from the substantial amount of inorganic Cl. The method developed has been found to be sufficiently precise (repeatability < 10% RSD) and accurate (recovery between 95 and 105%) and shows a linear dynamic range ($R^2 > 0.990$) from the limit of quantification (0.05 and 0.01 mg/L for Cl and Br in blood plasma, respectively) to at least 5 and 1 mg/L for Cl and Br, respectively. Quantification via either external or internal standard calibration provides reliable results for both elements. As a proof-of-concept, human blood plasma samples from a clinical study involving a newly developed Cl- and Br-containing active pharmaceutical ingredient have been analysed and the total drug exposure has been successfully described. Cross-validation has been achieved by comparing the results obtained on Cl- and on Br-basis.

This project is currently focusing on method development for quantitative metabolite profiling of Cl-containing drugs via a combination of reverse-phase (RP) HPLC for the separation of drug metabolites and ICP-MS/MS for Cl monitoring. The effect of gradient elution on ICP-MS sensitivity has been systematically investigated, taking the most important chromatographic parameters – e.g., organic solvent composition, type of organic modifier, slope of gradient, flow rate, eluent additives – into account. The main system suitability requirements (e.g., tailing factor, theoretical plate number, system precision) have been assessed taking into account the expectations of pharmaceutical authorities, and a comparison with the values attainable using HPLC-UV will be provided. The final goal is the demonstration of the utility of HPLC-ICP-MS/MS in the context of real-life applications.

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[1] Balázs Klencsár, Eduardo Bolea-Fernandez, María R. Flórez, Lieve Balcaen, Filip Cuyckens, Frederic Lynen and Frank Vanhaecke; Determination of the total drug-related chlorine and bromine contents in human blood plasma using high performance liquid chromatography – tandem ICP-mass spectrometry (HPLC-ICP-MS/MS), *J. Pharm. Biomed. Anal.*, **124** (2016) 112-119.