

A NEW UHPLC-MS/MS METHOD FOR THE EVALUATION OF THE INTRACELLULAR PHARMACOKINETIC OF THE NEW DIRECT ANTIVIRAL AGENTS (DAA) IN HCV POSITIVE PATIENTS SAMPLES

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Background: In the last few years, the development and introduction of the new direct antiviral agents (DAAs), sofosbuvir, simeprevir (SMV), daclatasvir (DAC), ledipasvir (LDV) and the new formulation Viekirax (paritaprevir (PAR), ombitasvir (OMB) and Ritonavir (RTV)) together with dasabuvir (DBV) have significantly improved the treatment of HCV infection. The new therapies have a better virological response rates, a shorter treatment duration and convenient single-tablet formulations. Although the efficacy of new regimens is very high, adverse events and treatment failure are present. Few data are available about the pharmacokinetic of the new drugs in real patient samples. Since these drugs explicate their activity inside cells, it could be useful to evaluate their concentration in the intracellular compartment. PBMC may represent a valid and easier available surrogate to hepatocyte cells. Up to now, few methods are reported to quantify these drugs in human plasma (none for all drugs simultaneously), and any method is available for intracellular quantification.

The aim of the study is to develop an UHPLC-MS/MS method for the evaluation of the intracellular concentrations and pharmacokinetic of the new DAAs.

Method: A gradient of ammonium-acetate 5mM pH9,5 and acetonitrile at a flow rate of 0.4ml/min was used for the chromatographic separation. Detection was carry out with a triple-quadrupole-tandem mass spectrometric coupled with electrospray ionization (ESI). The ESI was set in positive mode except for GS-331007 and for dasabuvir. PBMC samples were treated with acid phosphatase for GS331007 (sofosbuvir metabolite) analysis. Calibration curve and quality control were prepared using PBMC from healthy donors spotted with standards. Quinoxaline, DAC-D8, OMB-D6 were used as internal standards.

We analyzed 38 plasma and PBMC samples at 1 day from starting treatment and 189 samples at one month of therapy.

Results: Accuracy and precision inter and intraday were below 15% as required by FDA guidelines. Recovery was above the 50% for all drugs. Mean corpuscular volume of each PBMC sample was used to calculate the intracellular concentration of drugs.

We observed an high variability for each drugs in the intracellular concentrations and in the ratio between plasma and PBMC concentrations. We analyzed patients after 1 month of therapy and all drugs seem to accumulate into the cells, in particular LDV and OMB.

Mean and median values of the ratio PBMC/intracellular concentrations of different drugs at one month are respectively: 7.43; 4.60 for GS-331007 (sofosbuvir was not detectable in plasma nether

in cells), 3.47; 2.51 for SMV, 5.86; 4.12 for DAC, 22.19; 16.92 for LDV, 53.14; 34.07 for OMB; 4.66; 3.77 for DBV and 2.1;1.95 for PAR.

For all drugs except for OMB and DBV we saw a significant correlation between plasma and PBMC concentrations. Indeed, data showed a trend of increasing intracellular accumulation between one day and one month of therapy.

Conclusions:

The developed method is fast, robust and fully validate according to the FDA guideline. Intracellular concentrations are calculated considering the MCV, allowing a more accurate assessment of the drugs amounts.

Data about intracellular concentrations could lead to a greater knowledge of HCV drugs pharmacokinetic, helping in the management of HCV therapy and in the understanding of the toxicity and adverse events outcoming. For the future it will be usefull to increment the number of sample analyzed to evaluate the accumulation rates during the time of therapy (from 1 day to almost 1 month) and to observe the timing of drug elimination from cells at the end of treatment. Since we observed differences in intracellular concentrations among patients following the same therapy, we can suppose some drug-drug interactions and/or an influence by mutations of genetic polymorphisms involved in drug metabolism and penetration. This could be a purpose of investigation for future studies.