
ULTRAFAST AND HIGH-THROUGHPUT ANALYSIS OF ANTIRETROVIRAL DRUGS WITH MATRIX ASSISTED LASER DESORPTION/IONIZATION-TANDEM MASS SPECTROMETRY (MALDI-MS/MS)

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A new emerging mass spectrometric technique applied for ultrafast and high-throughput analyses of antiretroviral drug concentrations in blood and peripheral blood mononuclear cells (PBMCs) from HIV infected persons is presented. The mass spectrometric instrumentation consist of a matrix assisted laser desorption/ionization (MALDI) source containing a high rate repetition laser (1000 Hz) coupled with a triple quadrupole mass spectrometer (QqQ). The advantage of the application of MALDI source with a high repetition rate laser lies not only in a high sample throughput but also in a higher number of recorded accumulated spectra, this in contrast to regular MALDI sources with lower repetition rate lasers. A higher number of accumulated mass spectra increases the mass spectrometer's reproducibility considerably and therefore makes this type of MALDI source extremely suitable for quantitative analyses. Obtained relative standard deviations of presented mass spectrometric technique were lower than 15 %, which fully accomplish the FDA regulations for the application of bio-analytical methods. The new mass spectrometric technique omits a liquid chromatographic separation of samples prior to mass spectrometric detection. Samples are mixed with the MALDI matrix solution and 1 µL of the sample/matrix solution is spotted on to 96-or 384 well targets plates, the analysis time of one spot (one sample) is less than 10 seconds making it possible to apply this new mass spectrometric technique for ultrafast and high-throughput analyses. The total analysis time of a complete 384-well MALDI plate is approximately 30 minutes. Antiretroviral drug concentrations in PBMC as well in blood samples were quantified by means of multiple reaction monitoring (MRM) as we have published previously (van Kampen et al., 2008). Quantification limits of antiviral drugs such as the protease inhibitor drugs Lopinavir and Ritonavir were in the lower femtomole concentration level. We also investigated the possibility of quantification of antiretroviral drugs in blood by the application of the dry blood spot sample (DBS) technique. Whole blood from HIV infected patients was spotted onto paper special developed for DBS analyses and antiviral drugs were extracted from this paper by methanol and extracts were further processed with solid phase extraction (SPE) and antiviral drug concentrations were measured by the new MALDI-tandem mass spectrometric technique. Using this approach we are able to measure in patient samples antiretroviral HIV protease inhibitors and nucleotide analogue reverse transcriptase inhibitors (NRTI) such as Tenofovir and its bio-active metabolite Tenofovir-diphosphate in clinically relevant concentrations.

Reference

van Kampen, J. J., Burgers, P. C., et al. (2008) "Quantitative analysis of antiretroviral drugs in lysates of peripheral blood mononuclear cells using MALDI-triple quadrupole mass spectrometry" *Anal Chem* 80(13): 4969-75