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Analysis of highly cationic cell-penetrating peptides by hyphenated HPLC methods

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Research in novel drug delivery approaches includes successful application of peptide-based vectors, e.g., cell-penetrating peptides (CPPs) as carriers for macromolecular drugs such as insulin. Fluorescent probes are widely used for qualitative and quantitative determination but may lead to false negative or false positive results due to their large size and hydrophobic nature compared to the highly cationic hydrophilic CPPs. By introducing the very small 19F labels at one or more sites in a carrier peptide or therapeutic peptide cargo, it will be possible to evaluate the fate of the carrier. The aim of the current study is to develop a hyphenated HPLC-HRMS-SPE-19F NMR technology for elucidating CPP uptake and degradation pathways. Peptides based on penetratin and octaarginine were obtained by Fmoc-based solid-phase peptide synthesis. Peptides were initially analyzed by RP-HPLC using different, columns and mobile phases containing trifluoroacetic acid (TFA) and/or formic acid (FA). Furthermore, ion-suppression was tested to identify an MS-compatible mobile phase. Trapping efficiency was evaluated by comparing different SPE cartridge sorbent materials. Highly cationic peptides can be analyzed by LC-MS using a mobile phase containing 0.01% TFA providing acceptable chromatography and only slightly more ion suppression than 0.1% FA, although not ideal for octaargine. Peptides were most efficiently trapped on C18, C8, GP (polydivinyl-benzene) and mixed mode cation exchange SPE-cartridges. Overall the final optimized conditions for HPLC-UV-MS-SPE included a column from Supelco (Discovery BIO Wide pore C18, 150 × 4.6 mm, 300 Å, 3 µm) an MS-compatible mobile phase containing 0.01 % TFA and a silica based C18 cartridge for trapping.

Figure1: *The hyphenated analytical technique (HPLC-UV-MS-SPE-NMR)*

Biography

Malene V Christensen graduated from the University of Copenhagen at the Department of Chemistry in 2012. From 2013-2014 Malene was employed as Research Scientist at LEO Pharma at the Department of Early ADME, planning, performing and reporting physical chemistry studies upon working with HPLC/UPLC-UV and UPLC-MS. She is a third year PhD student from University of Copenhagen, Denmark at the Department of Drug Design and Pharmacology. Her work is focused on Development, Validation and use of a unique hyphenated HPLC-HRMS-SPE-19F NMR Technology for Elucidation of CPP degradation pathways.

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