Conference abstract PO-55

Analysis of *Citrullus colocynthis* Cucurbitacine Derivatives with HPLC-SPE-NMR

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Sci Pharm. 2009; 77: 254 doi:10.3797/scipharm.oephg.21.PO-55

Cucurbitacines are well know for their bioactivities and toxicity [1]. From the analytical point of view, this class of triterpene derivatives holds some challenges, due to their structural similarity and the presence of glycosides.
In *Citrullus colocynthis*, the coloquint, a series of strucurally related glucoside derivatives, the cucurbitacines E, I, J, K, and L are present. These are solely differentiated by subtile changes in the C-8 sidechain. With classical phytochemical approaches, this analyte class is well tackleable. However, the isolation of mg amounts needed for conventional NMR based structure characterization requires a remarkable investment of manpower, lab-time and consumables [2]. Hence, minimizing the analyte amount needed for identification (without loosing the required chemical information) is a major goal of modern phytochemistry. This goal has been recently realized by the HPLC-SPE-NMR platform allowing to obtain NMR information from µg amounts of analytes [3]. Combining the analytical HPLC based separation of the cucurbitacines with the trapping capabilities and the structural characterization power of the SPE-NMR device enabled the recording of all conventional 1D and 2D homo- and heteronuclear correlation spectra of *Citrullus* cucurbitacines from a crude methanolic extract of the plant material. The timeframe for recoding a complete data set ranged between 18 and 24 hours and is comparable to off-line data aquisition times of 4–5 mg sample material. Comparison of different SPE stationary phases showed the superior performance of the C-2 material and the GP (general purpose) resin, whereas C-8 and C-18 phases were of limited usability. Our data demonstrate, that HPLC-SPE-NMR is a valuable analytical platform with a broad applicability. The trap and release process of the online SPE device linking the HPLC and NMR instruments needs careful optimization of at least each analyte class investigated.

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