B009 Quantitative evaluation of coumarins from HPLC data without reference substances

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HPLC is the most common method used for the analysis and quantification of coumarins in plant extracts. The detection is usually made by UV at 254 nm or 310 nm (1, 2) or by multiple wavelength absorption with a diode-array detector (DAD) (3). Quantification is achieved using reference substances if available, which is often not the case. Due to the high variability of the specific absorptions ($A_{1\%,\ 1cm}$) of the different coumarins, normalisation (peak area percentages) is not exact enough. Therefore, in the course of our chemotaxonomic investigations of coumarins in Apiaceae fruits an evaluation method was worked out providing a formula which can be applied independently of reference substances and which allows the quantitative calculation of all known and unknown coumarins within plant extracts from one HPLC run. For the separation of the coumarins HPLC (RP-18, methanol-acetonitrile-water, gradient) was used. DAD detection and a sufficient peak separation are the prerequisites for the successful application of this simple method of calculation. The formula is as follows:

$$c\ (mg/ml) = (F / w) \cdot (A_{max}/A_{250}) \cdot (1 / v) \cdot 1.989 \cdot 10^{-5}$$

c (mg/ml) = content of a coumarin in the test solution; F = peak area [mAu · sec]; w = peak width [min]; A_{max} = highest absorption within the spectrum [mAu]; A_{254} = absorption at 250 nm within the spectrum [mAu]; v= volume of injection [µL].
The conversion factor (1.989×10^{-5}) was calculated from HPLC data of the furocoumarin xanthotoxin and is based on the assumption that the ratio of the highest absorption of a compound and its absorption at wavelength 250 nm is a constant and therefore independent of the concentration of the compound in the extract. The paper presents the causality and the deduction of the above-mentioned formula. Furthermore an example will be given calculating the composition of the coumarin fraction of *Heracleum mantegazzianum* fruits with 6 furocoumarins.

References: 1. C.A.J. Erdelmeier et al. (1985) J. Chromatography 346: 456-460. **2.** M.A. Hawryl et al. (2000) J. Chromatography A 88: 75-81. **3.** H. Vuorela et al. (1989) Planta Med: 55, 181-184.

B010 Monoclonal antibodies against oleanolic acid – a tool for a novel strategy in herbal drug screening

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Recently we reported on generation and characterisation of monoclonal antibodies against furanic labdane diterpenes (1). The methods were now adapted to produce monoclonal antibodies against oleanolic acid. This compound is a basic structural part of saponins in numerous medicinal plants which are known to exhibit a great variety of pharmacological effects. Total estimation of saponins is though difficult due to the limitations of detection methods, which normally refer to biological effects or analysis of a single compound.
In order to create an antigen suitable for production of antibodies oleanolic acid had to be coupled to a protein carrier. Thus, oleanolic acid was conjugated with BSA or thyroglobulin either directly or via succinic acid. The oleanolic acid-protein conjugates were used for immunisation of Balb/c mice. According to the methods developed by Köhler and Milstein (2) hybridoma cells were established. Cell-lines producing monoclonal antibodies were selected by ELISA.
Up to now four immunisations were performed. Screening of primary hybridoma cell lines resulted in selection of more than forty cell lines for further characterisation. Three cell lines were characterised in detail using a set of more than twenty triterpenes which were tested in competitive ELISA. For example, specificity of monoclonal antibodies produced by cell-line 10F10 is directed towards structural features of rings A and B. It was shown that the monoclonal antibodies are suitable to recognise the target structure also in crude extracts of herbal drugs.
One application of these monoclonal antibodies will be a target-structure orientated screening of plants from the Brazilian Atlantic Forest. Using this screening system the detection of plants rich in triterpenes will not depend on indirect physical effects, analysis can be performed directly in the fields and even less stable compounds will be detected.

References: 1. Brand, K. et al. (2001) GA annual meeting, Erlangen, Germany. **2.** Köhler, G. and Milstein, C. (1975) Nature 256, 495-497.