B039 Optimization of furanocoumarins HPLC separation occurring in *Heracleum sibiricum* L. by Drylab software

A. Bogucka-Kocka [a] and M. Hawryl [b]

[a] Department of Pharmaceutical Botany, Medical University, 4 Staszica, 20-081 Lublin, Poland. [b] Department of Inorganic and Analytical Chemistry, Medical University, 6 Staszica, 20-081 Lublin, Poland.

Furanocoumarins commonly occurring in Apiaceae family have been in the leucodermy and psoriasis therapy for many years(1). For this reason the plant materials containing psoralen derivatives – the source of these compounds - are the subject of investigations. One of the species containing number of these substances is *Heracleum sibiricum* L. commonly growing in central Europe (2).

The chromatographic separation of furanocoumarins is a difficult methodological problem because of their closely related structures (e.g. positional isomers) and similar retention behaviour. For this reason the furanocoumarins separation has to be performed by computer aided optimization. The aim of our work was the search of possibilities for the separation of the *H. sibiricum* L. extract by RP-HPLC using the gradient elution methods. The gradient profile was found by use of Drylab for Windows computer program. The retention data from two gradient separations (5-100% of methanol or tetrahydrofuran or acetonitril in water during 20 min and 5-100% methanol or tetrahydrofuran or acetonitril in water during 60 min) were set in the Drylab program to found the optimal gradient profile. The experiments were performed using LC-20 Shimadzu liquid chromatograph equipped with detector SPD-10 A-UV/Vis (λ=254 nm), pump LC-10 AT, column oven CTO-10 AS and controlled by SCL-10 A program. Stainless steel column Supelcosil LC-18, length 150 mm, Ø 4,6 mm (Supelco, USA) packed with 5mm particles was used in the experiments.

The complete separation by RP-HPLC using the gradient elution with acetonitril/water of seven coumarins: bergapten, ksantotoxin, izopimpinellin, heraclenin, imperatorin, byakangelicol and byakangelicin, components of *H. sibiricum* L., was obtained for the first time.

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B040 Characterization of betacyanins from *Hylocereus polyrhizus* (Weber) Britton & Rose

F.C. Stintzing, A. Schieber and R. Carle

Hohenheim University, Institute of Food Technology, Section Plant Foodstuff Technology, Garbenstrasse 25, D-70599 Stuttgart, Germany.

Cactus fruits and red beet are food sources rich in betalains (1). While the betalain profile of red beet is well known, data on the pigment pattern of cacti are comparatively limited. Very recently, the pigment pattern of the red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose has been reported for the first time (2, 3). In the present study, methods for betacyanin characterization and colour evaluation for *H. polyrhizus* pigments are described.

Removal of both mucilages and sugars permitted gentle pigment concentration. Alkaline, acid and enzymatic hydrolysis of betacyanins were used for their characterization. Betacyanins, betacyanidins and organic acids released through hydrolysis were monitored using HPLC-DAD. By electrospray ionization mass spectrometry assignment of eight betacyanins was possible. CIEL*a*b* values were recorded at pH 6.5 in McIlvaine buffer.

By β-glucosidase activity, three pigments were cleaved, thus indicating their glycosidic nature. Acid hydrolysis yielded betanidin and isobetanidin, whereas alkaline treatment resulted in malonic and 3-hydroxy-3-methyl-glutaric acids together with betanin and isobetanin. Therefore, all *H. polyrhizus* pigments were ascribed to the betanin-type. Further characterization was performed by HPLC-DAD-MS analyses. All betacyanins showed identical visible maxima, but lacked absorption indicative of cinnamic acid substitution at 310 nm. Bougainvillein r-I, betanin and isobetanin were found to constitute the non-acylated betacyanins of *H. polyrhizus*, whereas phyllocactin, hylocerenin, their corresponding C_{15} isomers together with a hitherto unidentified betacyanin were acylglycosides amounting to 75% of total pigments. The colour of *H. polyrhizus* was characterized by high chroma and a purple tonality.

H. polyrhizus is a promising source for colouring food, drugs and cosmetics. Besides their attractive appearance, betacyanins exert antioxidant activities with a high affinity to biological membranes (4). Therefore, cactus fruits are especially valuable for future food and health applications.

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