SL23 A subcutaneous microdialysis method combined with ESI LC–MS analysis for studying the skin penetration of tryptanthrin in Isatis extracts

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Isatis tinctoria L. (woad, family Brassicaceae) is an old European and Chinese dye and medicinal plant with a well documented history as an anti-inflammatory. Oral as well as topical application has been described in the ancient herbals. The anti-inflammatory potential of woad was recently confirmed in a broad pharmacological screening and the alkaloid tryptanthrin identified as an active principle with potent inhibitory properties on COX-2 and 5-LOX catalyzed eicosanoid synthesis (1, 2). Topical application of *Isatis* extracts in the TPA-induced ear oedema model in mice recently confirmed a significant anti-inflammatory effect *in vivo*. In view of a clinical study of *Isatis* extracts in topical application, analytical tools were needed for a suitable monitoring of the skin penetration of active principles in woad extracts.

Skin microdialysis allows for a time-resolved determination of local drug concentrations in volunteers (3). We established and validated a method for tryptanthrin using pig foreleg as a model. Microdialysis was carried out with a hollow fibre (i.d. 200 µm, exclusion limit 5000 amu) placed in the dermis at 1 to 1.5 mm below the skin surface. The flow rate of the dialysis fluid was 2 µl/min. Defined solutions of tryptanthrin and woad-extracts were applied onto the skin area above the fibre. Tryptanthrin concentrations in the dialysate were determined by ESI LC-MS, using d_8-tryptanthrin (4) as internal standard. A short, narrow-bore HPLC column (Purospher C-18 endcapped, 3 µm, 55 x 2 mm i.d.) was used without eluent split and with detection in the SIM mode (LOD: 100 pg; LOQ: 500 pg). In the pig forleg model, measurable tryptanthrin concentrations were found in the dialysate already 20 min after topical application of test compound or extract. Curves were recorded for 4,5 h. Depth of the fibre and amount of tryptanthrin affected the concentrations in the dialysate. Tryptanthrin penetration from extracts was proportionally higher than when a solution of pure compound was applied. Other extract substances may thus enhance the penetration of the poorly soluble alkaloid.

References: 1. Danz, H. et al. (2001) Plant. Med. 67: 411-416. **2.** Danz, H. et al. (2002) submitted. **3.** Schnetz, E. et al. (2001) Eur. J. Pharm. Sci 12(3): 165-174. **4.** Oberthür, C. et al. (2002) Pharmazie, in press.

SL24 Development of analytical methods for the biosafety assessment of genetically modified organisms

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Due to the lack of scientific knowledge, the use of genetically modified organisms (GMO) in the food industry is a major subject of controversy. Very recently, the concept of substantial equivalence of antinutrients was given (1) to regulate the introduction of novel food to the European market opening the door to a new analytical challenge: how to evaluate the biosafety of genetically modified organisms? In this context, the development of new techniques and methods of analysis for the collection of further comparative data through fingerprinting (metabolome) and quantitative determination of plant secondary metabolites is of main concern in order to determine whether GMO constitute a risk to human health or the environment. Choosing the potato tuber as a model of study, different genetically modified Bintje commercial potato variety were grown both in greenhouse and in the field in order the increase resistance to late blight (*Phytophtora infestans*). A HPLC/UV method was developed to quantify α-solanine and α-chaconine, the two major alkaloids found in potato tubers and well known for their toxicity. A preliminary screening obtained with greenhouse grown potato tubers indicated significant quantitative variations of both α-solanine and α-chaconine between the original Bintje potato and the genetically modified ones. In order to assess such results in a natural environment, genetically modified and unmodified Bintje potato variety were grown in the field together with other commercial potato varieties. A LC/DAD-UV/MS method was developed using the dichloromethane potato extract in order to obtain a fingerprint of the lipophilic constituents. Comparison of the fingerprints of genetically modified and non modified potatoes showed significant quantitative differences of some metabolites. Identification of these metabolites as well as quantification of α-solanine and α-chaconine in these potatoes is on course.

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