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Skin penetration behaviour of sesquiterpene lactones from different *Arnica* preparations using a validated GC-MSD method

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Abstract

Preparations of *Arnica montana* L. are widely used for the topical treatment of inflammatory diseases. The anti-inflammatory activity is mainly attributed to their sesquiterpene lactones (SLs) from the helenalin and 11α,13-dihydrohelenalin type. To study the penetration kinetics of SLs in *Arnica* preparations, a stripping method with adhesive tape and pig skin as a model was used. For the determination of SLs in the stripped layers of the stratum corneum (SC), a gas chromatography/mass spectrometry method was developed and validated. Thereby the amount of helenalin derivatives was calculated as helenalin isobutyrate, and 11α,13-dihydrohelenalin derivatives as 11α,13-dihydrohelenalin methacrylate. This GC-MSD method is suitable also to determine low amounts of SLs in *Arnica* preparations.

The penetration behaviour of one gel preparation and two ointment preparations was investigated. The SLs of all preparations show a comparable penetration in and a permeation through the stratum corneum, the uppermost part of the skin. Interestingly, the gel preparation showed a decrease of the penetration rate over 4 h, whereas the penetration rate of ointments kept constant over time. Moreover, we could demonstrate that the totally penetrated amount of SLs only depends on the kind of the formulation and of the SLs-content in the formulation but not on the SLs composition or on the used extraction agent.

Keywords: *Arnica montana*; Herbal medicines; Sesquiterpene lactones; Skin penetration; Gas chromatography; Mass spectrometry; Validated method

1. Introduction

Preparations of *Arnica montana* flowers have been used in traditional medicine for topical treatment of post-trauma effects and inflammatory diseases [1]. Sesquiterpene lactones (SLs) of the 10α-methylpseudoguaianolide-type, such as helenalin and 11α,13-dihydrohelenalin esters, are the active components which mainly mediate the anti-inflammatory effects [1]. Two chemotypes of *A. montana* can be differentiated: the central European chemotype with dominance in helenalin esters and the Spanish chemotype with almost 11α,13-dihydrohelenalin esters [2]. Both chemotypes are used in commercial products. Alcoholic as well as oily extracts are prepared and used in gels, cremes, ointments or as Arnica oil.

Using a stripping method with adhesive tape and pig ear skin as a model [3–5], we have recently investigated the penetration kinetics of SLs in alcoholic Arnica preparations and in an Arnica gel [6]. We could show a sufficient penetration of SLs into and a permeation through the upper layer of the skin, the stratum corneum (SC), which is regarded as the main barrier and the greatest obstacle to transdermal diffusion [7]. Moreover, we demonstrated that isolated SLs being dissolved in an alcoholic solution permeated to a lower rate through the SC than SLs being in an alcoholic extract, indicating the advantageous use of plant preparations. Pig skin is the most suitable model for human skin because of similarities in the epidermal composition, dermal structure, lipid content, histochemistry and general morphology [3,8] as well as permeability behaviour [9].

In commercial products, oily extracts from Arnica flowers are also used in the form of ointments. Continuing our penetration studies, we therefore compared the penetration behaviour of SLs from a gel with that of an ointment. For a better comparison, we studied in both cases Arnica products prepared from flowerheads of the Spanish type. Two different incubation times were considered. Additonally, the correlation between the SLs

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content and the penetration rate was investigated using an oily extract enriched with SLs.

For the quantitative determination of SLs occurring in low amounts a selective and sensitive analytical method is necessary. Based on the established GC-FID method [2] which is not sufficient enough, a GC-MS analytical method was developed and validated enabling quantification of helenalin and 11α,13-dihydrohelenalin esters at low concentrations.

2. Experimental

2.1. Materials

Three different Arnica preparations were investigated: the gel preparation “Kneipp Arnika KühlGel®” (Ch.-B.: 0119656; preparation B), the ointment “Arnica-Kneipp Venensalbe®” (Ch.-B.: 0305895; preparation A) as well as an ointment enriched with additional SLs (Ch-B.: ASS37; preparation C). Preparation C is identical with A, but an Arnica CO_2-extract was finally added resulting in a 10-fold SL-content compared to preparation A. The composition of the preparations is given in Table 1. All preparations were manufactured by Kneipp Werke (Würzburg, Germany). Preparation B is a commercially available product, preparation A is now commercially available under the designation “Kneipp Arnika Salbe S”.

11α,13-Dihydrohelenalin acetate and helenalin isobutyrate were isolated from flowerheads of *A. montana* as previously described [2]. Identity was confirmed by NMR and MS analysis, and purity was evaluated by GC and TLC analysis.

The used organic solvents and sodium sulphate (anhydrous) were of analytical grade and obtained from Merck (Darmstadt, Germany). Sephadex LH-20 was purchased from Pharmacia Biotech (Uppsala, Sweden) and Santonin from Sigma (St. Louis, USA).

2.2. GC-MSD system

GC analyses were carried out with an HP6890 series GC-system (Hewlett–Packard, Wilmington, USA) using helium 5.0 as carrier gas. A fused silica capillary column (25 m × 0.25 mm i.d.) coated with 0.25 μm dimethylsiloxan was used (Optima 1, Machery-Nagel, Düren, Germany). The flow rate was set to 1.0 mL/min. The temperature profile started at 120 °C followed by a rate of 10 °C/min to 270 °C, which was held for 20 min. The injector and detector temperatures were 290 °C, the injection volume was 1.0 μL and was not split.

An Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, USA) at ionisation energy of 70 eV was used. Identification of SLs in the quantitative analyses was achieved by holding the EI mass spectra between 40 and 400 amu. A sim mode was used at 246 amu (santonin and dihydrohelenalin derivatives) and 244 amu (helenalin derivatives) for quantification.

2.3. Validation parameters and procedures

Validation included tests on specificity, calibration curve, precision, accuracy and stability. Two matrices were used: one matrix obtained from an ointment according to the processing mentioned below and one matrix obtained from strips loaded with horny-layer according to the processing described below in the “skin penetration study” chapter. The composition of the used placebo ointment was identical to preparation A, but contained pure vegetable oil instead of an Arnica oil extract.

2.4. Determination of the SLs content in Arnica preparations

Extraction of SLs from ointment: 5.0 g ointment and 0.20 mg santonin (internal standard) were suspended in 25.0 mL acetone. After addition of 10 g sodium sulphate (anhydrous), extraction was done for 5 min. After a period of further 5 min, the solution was filtered. The residue was extracted again with 15 mL acetone and filtered. This extraction was repeated four times. The combined solutions were reduced to a volume of about 20 mL, 10 g sodium sulphate (anhydrous) was added, stirred for 5 min and filtered. Flask and filter were rinsed with acetone. The combined solution was evaporated. The clear, yellow oily residue was dissolved in 10 mL petroleum benzine (boiling range 40–60 °C) and extracted four times with 15 mL ethanol (65%, v/v). A waiting period of 10 min followed after each extraction period. The combined ethanolic solutions were evaporated and dissolved in 4 mL acetone, and put on a conditioned (2 mL acetone) solid phase extraction column (Chromabond® NH_2 6 mL/500 mg

Table 1
Composition (%, m/m) of the Arnica preparations A (Arnika Kneipp VenenSalbe®) and B (Kneipp Arnika Kühlgel®)

Preparation A Arnika Kneipp VenenSalbe®		Preparation B Kneipp Arnika Kühlgel®	
Water, demineralized	68.00	Water, demineralized	50.32
Arnica oil extract (1:3.5–4.5)	10.00	Arnica tincture (1:10)	25.00
Glycerol 85%	n.s.	Ethanol 96.5% (v/v)	22.30
Stearic and palmitic acid	n.s.	Polyacrylic acid (Carbopol 980 NF)	n.s.
Soya lecithine	n.s.	Tris amino ultra pure	n.s.
Emulsifying cetylic-stearic-alcohol (Lanette N®)	n.s.	Perfume oil	n.s.
Cetylic alcohol (Lanette 16)	n.s.	Camphor	n.s.
Glycerol monooleat	n.s.		
Benzyl alcohol	1.00		

n.s.: Not specified.