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In Vitro and *In Situ* Characterization of Triterpene Glycosides From *Cimicifuga racemosa* Extract

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ABSTRACT

Cimicifuga racemosa products are widely used in the treatment of climacteric symptoms. The aim of this study was to evaluate *C racemosa* extract Ze 450 according to Biopharmaceutics Classification System (BCS). Triterpene glycosides served as analytical marker and were evaluated for solubility and absorption properties. pH-dependent thermodynamic solubility was tested via shake flask method, and dissolution performance of a herbal medicinal product containing *C racemosa* extract Ze 450 was assessed. Absorption was estimated by *in vitro* permeation through Caco-2 monolayers. Furthermore, different intestinal segments were screened for absorption performance using an *in situ* rat model. Over a physiological pH range, triterpene glycosides exhibited pH-dependent solubility with highest concentration at pH 7.5. Dissolution profiles showed rapid dissolution of actein and 23-*epi*-26-deoxyactein. Furthermore, 23-*epi*-26-deoxyactein as surrogate for contained triterpene glycosides showed a high permeability through Caco-2 monolayers. Results of *in situ* rat model showed absorption capacity for 23-*epi*-26-deoxyactein in duodenum, jejunum, ileum, and colon. The results indicate high bioavailability of triterpene glycosides from *C racemosa* extract Ze 450. With regard to BCS, triterpene glycosides can be classified into BCS class I (high solubility, high permeability).

Introduction

Products containing *Cimicifuga racemosa*, also known as *Black Cohosh*, gained great popularity in the treatment of climacteric symptoms. Several randomized, controlled clinical studies proved efficacy and safety in the indication of menopausal symptoms.[1-4] Committee of Herbal Medicinal Products of the European Medicines Agency lists the well-established use of *C racemosa* (L.) Nutt. rhizome extracts for the treatment of menopausal complaints such as hot flushes and profuse sweating.[5]

The aim of this study was to evaluate biopharmaceutical performance of *C racemosa* extract Ze 450 with special regard to Biopharmaceutics Classification System (BCS).[6] The rate and extent to which the active substance is absorbed from a drug product and becomes available at the site of action is dependent on release, solubility, absorption, and first-pass metabolism. BCS classifies substances according to their solubility and permeability into 4 classes (Table 1). To evaluate saturation solubility, pH-dependent thermodynamic solubility was determined using an *in vitro* shake flask method. As *C racemosa* products of Ze 450 established on the market are mainly solid dosage forms such as tablets, *in vitro* dissolution performance of a herbal medicinal product containing *C racemosa* extract Ze 450 was assessed. Absorption properties were estimated by *in vitro* permeation through Caco-2 monolayers. Additionally, different intestinal segments were screened for appropriate absorption performance using an *in situ* rat model.

Materials

C racemosa rhizome dry extract Ze 450 was provided by Max Zeller Söhne AG (Romanshorn, Switzerland). As herbal medicinal product, Cimifemin® forte containing 13 mg *C racemosa* rhizome dry extract Ze 450 from Max Zeller Söhne AG was used for dissolution testing. Actein, 23-*epi*-26-deoxyactein, cimiracemoside C, and

Abbreviations used: Ze 450, *Cimicifuga racemosa* extract; BCS, Biopharmaceutics Classification System; ab, apical-to-basolateral compartment; ba, basolateral-to-apical compartment; C_{max}, maximal plasma concentration; t_{max}, time when maximal plasma concentration was detected; AUC, area under the curve; AUC_{phys}, area under the curve related to length of intestinal segment and transit time; UPLC, ultra-high performance liquid chromatography; MS, mass spectrometry; ES+, MS ionization source in positive ion mode; m/z, mass-to-charge ratio; SIR, selected ion recording.

Conflicts of interest: The authors declare no competing financial interest.

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Table 1
Biopharmaceutics Classification System

BCS Class I	BCS Class II
High solubility High permeability	Low solubility High permeability
BCS class III	**BCS class IV**
High solubility Low permeability	Low solubility Low permeability

4-*O*-acetyl-hydroshengmanol-3-*O*-β-D-xylopyranosid were purchased form PhytoLab (Vestenbergsgreuth, Germany). Polysorbate 20 and polyethylene glycol 400 were obtained from Hänseler (Herisau, Switzerland). Caco-2 cells HTB-37 were obtained from ATCC (American Type Culture Collection, Manassas, VA). Dulbecco's modified eagle medium, nonessential amino acids, Gibco® GlutaMAX™ containing 200 mM L-alanyl-L-glutamine dipeptide in 0.85% NaCl, and penicillin/streptomycin were from Thermo Fisher Scientific (Reinach, Switzerland). Hank's balanced salt solution (HBSS) buffer, digoxin, sodium hydroxide solution, dimethyl sulfoxide (DMSO), acetonitrile, formic acid, propranolol hydrochloride, and fluorescein isothiocyanate-dextran (FITC-dextran) were obtained from Sigma-Aldrich (Buchs, Switzerland). All other chemicals were purchased from Merck (Darmstadt, Germany). Wild-type male Sprague Dawley rats from University of Heidelberg were used.

Caco-2 Cell Culture

For transport assay, Caco-2 cells were seeded on 0.336 cm^2 PET (polyethylene terephthalate) filters and 24-well plates with a density of 1×10^5 cells/cm^2. Caco-2 cells were used 21 days post-seeding in passage 67. The cells were maintained in Dulbecco's modified eagle medium supplied with 10% fetal bovine serum, 1% L-glutamine, 1% nonessential amino acids, and penicillin/streptomycin (10 mg/mL) at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Culture medium was changed every 2-3 days.

Animals

The *in situ* study was performed at the Department of Pharmacy and Molecular Biotechnology at University of Heidelberg. It was approved by the local authorities according to the guidelines of animal welfare. Wild-type male Sprague Dawley rats of 200-300 g weight were acclimatized and fasted overnight with free access to water.

Methods

As herbal extracts contain a complex mixture of different physicochemical constituents, analytical markers were selected. Actein, 23-*epi*-26-deoxyactein, cimiracemoside C, and 24-*O*-acetylhydroshengmanol-3-*O*-β-D-xylopyranoside served as surrogates for the main constituents of *C racemosa*, triterpene glycosides of the cycloartane-type aglycone.[7] Structural formulas are given in Figure 1. Triterpene glycosides were quantified in solubility samples as sum of all detected triterpene glycoside peaks including the listed analytical marker. Dissolution trials resulted in much lower concentrated samples, so a quantification was just possible for the main triterpene glycosides actein and 23-*epi*-26-deoxyactein. In Caco-2 assay samples and plasma samples, 23-*epi*-26-deoxyactein served as sole analytical marker as concentrations of other triterpene glycosides were below the quantification limit of the used method. For considering influence of other extract constituents on the performance of selected analytical marker, experiments were performed using Ze 450 in its entity.

pH-Dependent Thermodynamic Solubility

To determine pH-dependent thermodynamic solubility, shake flask method according to the study by Mishra and Yalkowsky[8] was used, where the test substance was shaken over a defined period of 48 h in a flask containing the test medium whereby a balance between solid substance (solute) and dissolved substance (solution) appear. Sodium phosphate buffers of pH 1.2, 2.2, 3.2, 4.5, 5.5, 6.8, and 7.5 were prepared according to United States Pharmacopeia and European Pharmacopoeia (EP) recommendations.[9,10] An amount of 300 mg of *C racemosa* rhizome dry extract Ze 450 each was weighed out in 50-mL Erlenmeyer flasks. After adding 25 mL of the appropriate buffer medium, Erlenmeyer flasks were closed. Then, the flasks were shaken in a water bath Julabo SW 22 at 37°C (±1°C) and 90 rpm for 48 h. Samples of buffer solution were taken after 24 and 48 h by using a 5-mL syringe. Samples were filtered through a syringe glass filter (0.45 μm) by discarding the first milliliter of the filtrate. Three replicate

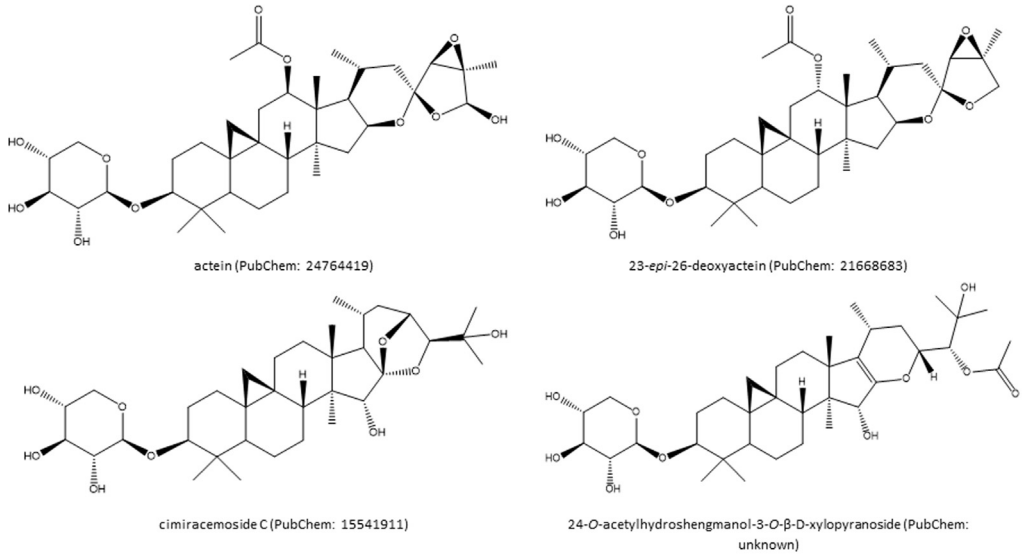

Figure 1. Structural formulas of triterpene glycosides selected as analytical marker.