

Thin layer chromatography (TLC) and multivariate data analysis of willow bark extracts

K. Wuthold^a, I. Germann^b, G. Roos^a, O. Kelber^c, D. Weiser^c, H. Heinle^b and K.-A. Kovar^a

^a Eberhard-Karls-University, Department of Pharmacy, Auf der Morgenstelle 8, 72076 Tübingen, Germany; kim.wuthold@uni-tuebingen.de

^b Eberhard-Karls-University, Department of Physiology, Gmelinstr. 5, 72076 Tübingen, Germany; Ines.Germann@uni-tuebingen.de

^c Steigerwald Arzneimittelwerk GmbH, Scientific Department, Havelstr. 5, 64295 Darmstadt; kelber@steigerwald.de

Willow bark is known as an anti-inflammatory, antipyretic and analgesic agent, and is particularly used for rheumatic diseases showing little side effects in contrast to chemically defined anti-inflammatory agents. According to the European Pharmacopoeia, 4th edition, 2002, the drug contains not less than 1.5 per cent of total salicylic derivatives, expressed as salicin.

But as the salicin content of willow bark extracts is not sufficient to predict their therapeutic effectiveness, other chemical components seem to significantly influence its pharmacological properties. To which degree these components are jointly responsible for the effectiveness is so far unknown.

Generally, conventional standardization of plant extracts by use of marker substances does not necessarily correlate with the effectiveness of the extract. According to the prevailing opinion, the effectiveness of plant preparations depends on the amounts of a complex mixture of components. High performance thin layer chromatography (HPTLC) is very well suited for separating and characterizing plant extracts. Therefore it could be advantageous to correlate HPTLC results and pharmacological properties by use of multivariate data analysis [1].

Chromatographic data of 22 various extracts of willow bark obtained by a TLC scanner that enables measurement of HPTLC tracks simultaneously in the range of $\lambda = 200\text{-}600\text{nm}$ were chemometrically evaluated and calibrated using the partial least squares algorithm. We correlated the spectroscopic pattern of the extracts with results of two chemiluminescence tests indicating their antioxidative properties: the AAPH [2,2'-azobis(2-amidinopropane)dihydrochloride] reaction which produces various species of radicals and the XOD (xanthine/xanthine oxidase) reaction which specifically generates superoxide anions [2][3]. The obtained models are confirmed by prediction of a small external test set of willow bark extracts.

Acknowledgements: J&M GmbH, Aalen, Germany.

References:

1. Wuthold, K., Roos, G., Simmen, U., Kovar, K.-A. (2003), Analytical Study of Extracts of St John's Wort (*Hypericum perforatum*), Evaluation of HPTLC Plates by Multivariate Data Analysis, *J. Planar Chromatogr.* 16: 15-18.
2. W. Schneider, P. Dalferth, O. Kelber, G. Friedemann, R. Haasis, H. Heinle (1999), Oxidizability of low density lipoprotein and total antioxidative capacity of plasma are differently altered during induction and regression of hypercholesterolemia in rabbits, *Atherosclerosis* 144, 69-72
3. W. Reimann (2003), PhD thesis, University of Tübingen, Faculty of Chemistry and Pharmacy