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Analytical and Phytochemical Investigations on Hypericin and Related Compounds of *Hypericum perforatum*

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Summary

This study is dedicated to questions concerning the quality control of products of *Hypericum perforatum*. It is divided into six chapters. Each of the chapters two to six starts with a literature overview on the subject examined, followed by the description of experiments, results, discussion, conclusions and references mentioned in the relevant chapter.

Chapter 1 presents factors influencing the quality of extracts of Hyperici herba and discusses tools allowing its evaluation. At present, most of the commercial extracts are standardized on a certain content of hypericin, as hypericin is a very characteristic constituent and as the active principle(s) and the mode of action of *Hypericum perforatum* have still not been clarified yet. This requires that hypericin is available as reference substance of constant high quality.

On the basis of this background, an isolation process for hypericin and also pseudohypericin has been developed, which is described in **chapter 2**. The isolation procedure starts with a liquid-liquid partition of a dry extract of Hyperici herba in a separatory funnel followed by High-Speed Countercurrent Chromatography. The identity and purity of the two substances were ratified by TLC, HPLC, UV/VIS spectroscopy, EIMS and NMR measurements.

Chapter 3 deals with the physicochemical properties of hypericin and pseudohypericin, which must be known to be able to assess influences of the reference substance on the quantification result. The evaluation of the absorbance data of hypericin and pseudohypericin revealed the ***molar/specific coefficients of absorbance*** in methanol-pyridine (99:1, v/v) at the maximum of the longest wavelength to be 51936/1030 and 43486/836, respectively. The absorbance data of hypericin were also determined in methanol. They were not significantly differing from those in the presence of pyridine. The decrease of the coefficients by water addition was found to be the same for hypericin and pseudohypericin. It was concluded that hypericin and pseudohypericin reveal the same ***homoassociation*** behavior. The ***solubility*** of hypericin turned out to be enhanced by the addition of pyridine. In pure methanol only 37.17 µg/ml could be dissolved. In comparison, 320.91 µg hypericin were soluble in one ml methanol-pyridine (99:1, v/v). Tests on the ***stability*** of hypericin and pseudohypericin in extracts and standard solutions were done under different temperature and light conditions monitored by VIS spectroscopy and HPLC-VIS / DAD measurements. All solutions were stable at -20 °C in darkness over the investigated time period of 140

days. Higher temperatures, light and the presence of pyridine turned out to accelerate the degradation of pseudohypericin, while exposure to light was most aggressive. Hypericin showed higher stability, light being the only factor investigated that decreased the concentration of hypericin. The instability in the presence of light was more pronounced in the extract solution both for hypericin and pseudohypericin. Under all the other storage conditions, the stability of pseudohypericin in the extract solutions was improved. Cyclopseudohypericin was assumed to be one of the transformation products of pseudohypericin.

Besides the reference substance, various other factors, not completely known yet, are affecting the evaluation of the content of hypericin in drug samples and dry extracts of Hyperici herba. Therefore, **chapter 4** examined, which effect quantification techniques, extracting solvents and extracting methods in general had on the results. In **section 4.1**, some HPLC methods were compared with each other, with VIS spectroscopy and TLC-densitometry using a commercial dry extract. The modified HPLC method of Kerb turned out to be most suited for the quantification of naphthodianthrones, as it showed the shortest run time, best selectivity and highest reproducibility. Applying this method, pyridine did not influence run time and quantification, allowing preparing standard solutions with pyridine and doing extraction without. VIS spectroscopic results were higher than HPLC results caused by differences in selectivity. The ratio VIS spectroscopic to HPLC results was not the same for all the HPLC methods investigated. A drug sample was subjected to various extraction methods in **section 4.2** revealing the method of the Ph. Helv. 8, which uses tetrahydrofuran-water (8:2, v/v) for extraction, to be the most efficient. The application of the method of the DAC (1986) showed preextraction with dichloromethane to diminish differences between the results of the two quantification techniques. This could also be seen in **section 4.3**. There, it was investigated in which degree the drug sample itself affected the results, finding the ratio of blossoms to leaves to play a crucial role. To clarify if the factors affecting the quantification of naphthodianthrones were different for drug material and commercial extracts, **section 4.4** looked closer at the extraction procedure of a commercial dry extract. Ultrasonic extraction turned out to be the fastest, most efficient and precise method to extract naphthodianthrones from the dry extract Ze117. It was shown that the ratio of VIS spectroscopic to HPLC results was higher for the extracting solvent methanol than for acetone. Chlorophyll and other compounds, not definitely defined yet, were made responsible for the discrepancy. Polarity and selectivity of the extracting