

## Pre-Extraction and Extraction of Hypericin in St. John's Wort (*Hypericum perforatum*) using the SpeedExtractor E-916

St. John's Wort herb and capsules with dry extract are widely used in herbal medicine. For quality control reason the amount of hypericin in the products is determined by extraction and photometric quantification. The quantification by photometry is interfered by co-extracted chlorophyll. Classically, chlorophyll is removed by a time-consuming manual procedure. A convenient removal of chlorophyll followed by quantitative extraction of hypericin is presented, based on pressurized solvent extraction using the SpeedExtractor E-916.

### Introduction

In herbal medicine St. John's Wort herb and capsules with dry extract are widely used for the treatment of depressions. In this products hypericin is determined for quality reasons. The determination of the total amount of hypericin can be done by extraction and photometric quantification at 590 nm. The quantification by photometry is interfered by co-extracted chlorophyll. Removal of the interfering chlorophyll was achieved by a pre-extraction with dichloromethane. The remainings were then extracted with methanol to quantify hypericin.

### Experimental

**Instrumentation:** SpeedExtractor E-916, Photometer: Thermo Helios, Ultrazentrifugal mill: Retsch, ZM 200 with distance sieve 0.5 mm.

**Samples:** Dried and cut St. John's Wort herb and capsules containing 425 mg dry extract of St. John's Wort

Approx. 0.6 g of the ground herb or approx. 0.05 g of the dry extract was mixed with quartz sand and extracted in two consecutive extractions with the SpeedExtractor using the parameters shown in Table 1. The samples were extracted in triplicate.

Table 1: Extraction method of the SpeedExtractor E-916

|                    | Pre-extraction  | Main-extraction |
|--------------------|-----------------|-----------------|
| Temperature        | 80 °C           | 80 °C           |
| Pressure           | 100 bar         | 100 bar         |
| Solvent            | Dichloromethane | Methanol        |
| Cells              | 10 ml           | 10 ml           |
| Vials              | 240 ml          | 240 ml          |
| Cycles             | 2               | 4               |
| Heat-up            | 1 min           | 1 min           |
| Hold               | 4 min           | 2 min           |
| Discharge          | 2 min           | 2 min           |
| Flush with solvent | 5 min           | 5 min           |
| Flush with gas     | 4 min           | 4 min           |

After completing to 200 ml, photometric quantification at 590 nm was done.

Absorption coefficient:  $E_{cm}^{g/100ml} = 870$

### Results

By performing a pre-extraction with dichloromethane the chlorophyll can be efficiently removed from the samples without affecting the hypericin content. Only a negligible amount of chlorophyll remains, with insignificant interference with hypericin at 590 nm (Fig. 1 and 2). Found

concentrations in herb and capsules correspond with the declared values (Table 2).

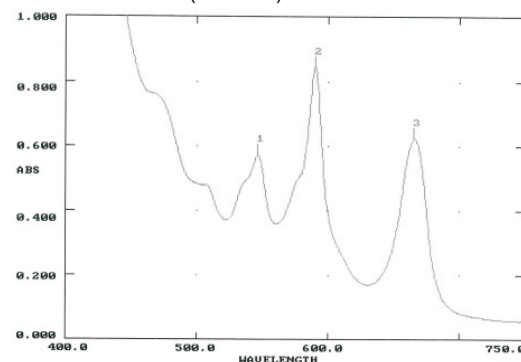


Figure 1: Spectrum without pre-extraction; 2: hypericin, 3: chlorophyll

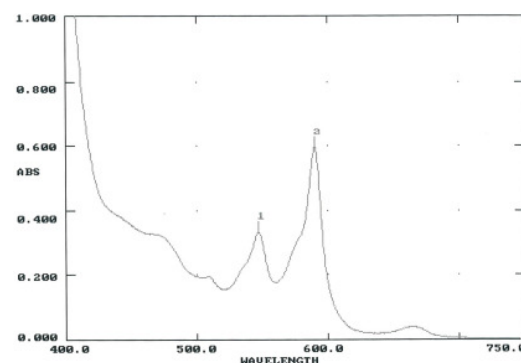


Figure 2: Spectrum with pre-extraction; 2: hypericin

Table 2: Determined content of hypericin, n=3

|          | Hypericin [rsd]           | Declared value of supplier |
|----------|---------------------------|----------------------------|
| Herb     | 0.8 mg/g [1.6 %]          | min. 0.8 mg/g              |
| Capsules | 1.18 mg / Capsule [1.9 %] | min. 0.75 mg / Capsule     |

### Conclusion

Application of two consecutive extractions is a fast and reliable way for the determination of total hypericin in St. John's Wort herb and capsules.

### References

- [1] PhEur 6.2, 07/2008:1438, Hyperici herba  
SpeedExtractor E-916 operation manual  
For more detailed information refer to Application note 015/2009