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# Physicochemical properties of harpagoside and its in vitro release from *Harpagophytum procumbens* extract tablets

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## Summary

**The objective of this investigation was to characterize the active-component harpagoside of *Harpagophytum* extract from a physico-chemical perspective and to determine its in-vitro release from tablets according to DAB 1996. It was found that both pure harpagoside and harpagoside in *Harpagophytum* extract have an octanol-water distribution coefficient of approximately 4 which is neither dependent on temperature nor on pH. The mean harpagoside content in *Harpagophytum* tablets of Batch 9102 was 16.4 mg (S.D. 0.2; S.E. 0.03). Related to a tablet weight of 365 mg (100%), this corresponds to a haragoside content of 4.5% (S.D. 0.049; S.E. 0.006). On average the tablets disintegrate after 18 ± 3 minutes (mean ± SD). The tablets taken from Batch 9102 released the active component harpagoside well, with a $t_{50}$ of 13.5 min, a $t_{90}$ of 23 min and a $t_{95}$ of 25 min in relation to 16.5 mg of harpagoside per dose. Harpagoside content decreased by about 10% in artificial gastric fluid within a period of 3 hours and remained stable in artificial intestinal fluid for a period of 6 hours.**

Key words: *Harpagophytum procumbens,* harpagoside, physiochemical properties, releuse from tablets, stability in gastric fluid.

## Introduction

An important aspect of general quality assays for all allopathic drugs is to determine the biopharmaceutical quality (Specker et al., 1978; Siewert, 1995). For example, it is required to investigate the release of the active ingredient of a drug using suitable in vitro methods and disintegration tests of tablets. Methods used include the disintegration tester as defined in the German Pharmacopea (DAB) or the rotating paddle US Pharmacopea (USP XXII) and the DAB, the rotating basket USP XXII/DAB or similar. As part of the quality assurance measures, the standards applying to allopathic drugs are increasingly used for herbal medicinal products, e.g. for preparations of ginseng (Keller 1997), however, not yet for *Harpagophytum* preparations (marker compound harpagoside).

A standardized extract of *Harpagophytum procumbens* has been shown to alleviate rheumatic pain (Chrubasik et al., 1996a). In-vitro experiments revealed that one of the active components, the iridoid glycoside harpagoside, inhibits dose-dependently both pathways of the eicosainoid biosynthesis, the cyclo-oxygenase and the lipoxygenase (Tippler et al., 1996) and offers, thus, a broader effect mechanism than the nonsteroidal anti-inflammatory drugs. The extract is more potent than the marker compound harpagoside (Tippler et al., 1996). However, recently it was shown that the clinical efficacy of the extract depends on the quantity of active ingredients with a higher daily consumed harpagoside input being more effective (Chrubasik et al., 1999). The objective of this investigation was to determine the octanol-water distribution coefficient of harpagoside and its in vitro release from tablets.

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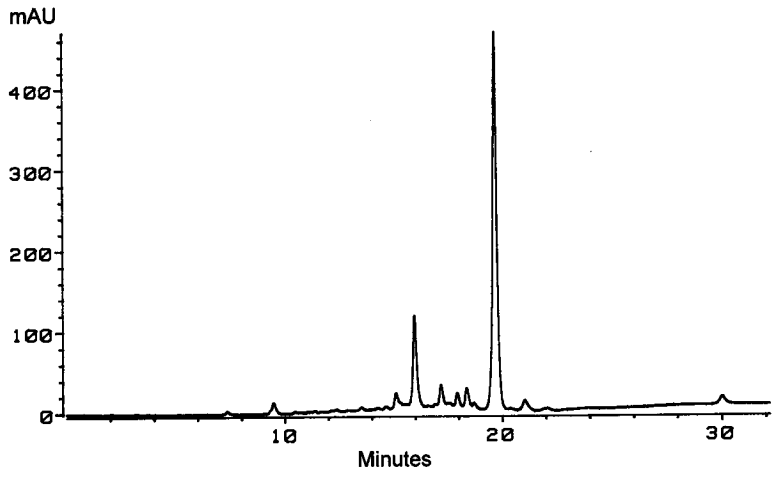


**Fig. 1.** HPLC-fingerprint of *Harpagophytum* extract
Column: LiChroCART 250-4, Merck, Darmstadt, RP 18, endcapped, 5 µm
Solvent A: water (+3 g phosphoric acid 85%/1000 g)
Solvent B: acetonitrile, ROTISOLV, gradient:

| min | A | B | min | A | B |
|---|---|---|---|---|---|
| 0 | 100 | 0 | 22 | 0 | 100 |
| 10 | 100 | 0 | 27 | 0 | 100 |
| 20 | 60 | 40 | 35 | 100 | 0 |
| | | | 40 | 100 | 0 |

flow rate: 1.3 ml/min, injection volume 20 µl, Detection 280 nm UVvis
The peak at rentention time 20 min corresponds to harpagoside.

## Methods

### Octanol-water distribution coefficient of harpagoside

Solutions with a pH of 2 to 9 were prepared mixing 0.1 N aqueous solutions of phosphoric acid, sodium phosphate, sodium hydrogen phosphate and disodium hydrogen phosphate, respectively. These solutions and n-octanol were then saturated in relation to each other. Twenty-five ml octanol and 25 ml phosphate buffer aliquots were shaken and incubated for 30 minutes in a water bath at 20 °C and at 37 °C, respectively. After adding 500 µl aqueous harpagoside (10 mg/ml) or an aqueous solution of *Harpagophytum* extract (50 mg/ml), the samples were incubated for 60 minutes and intensively shaken at 10 minute intervals. The samples were then centrifuged, and the harpagoside content of the lower and upper phase was analyzed with HPLC (UV detection 280 nm) using an established standard procedure (Chrubasik et al., 1996c).

### Investigations of Harpagophytum tablets

- *Tablet characterisation:* Batch 9102 involved white tablets with a compact extract core and a white coating. The tablets contained 200 mg extract WS 1531* (drug extract ratio 6 to 9:1, harpagoside enriched, minimum 5%) and silicium-dioxide, cellulose and lactose, magnesium-stearate, crosslinkend polyvidone and polyvidone as additives. The coating consisted of hydroxypropylmethylcellulose, macrogol, titanium-dioxide and talcum. The mean tablet weight was 365 mg (standard deviation 5.7). The mean harpagoside content was determined as previously described (Chrubasik et al., 1996c). In short, following a complete methanolic extraction HPLC analysis was carried out (see Fig. 1). The method was validated for batch 9102. For the intra-assay coefficient of variation 11, for the inter-assay coefficient of variation 12 samples were used. Recovery was determined for 10 mg, 15 mg and 20 mg harpagoside added to the batch samples.
- *Disintegration of the Harpagophytum tablets:* Using DAB/Disintegration Tester DAB, 5 tablets of Batch 9102 were incubated in water (30 strokes/min) at 37 °C until fully disintegrated. The resulting disintegration times were recorded.
- *In vitro release of harpagoside from Harpagophytum tablets:* The release tests involving the tablets of Batch 9102 were carried out according to DAB 1996 V.5.4. One tablet was placed in the release vessel containing artificial gastric fluid. After 5, 10, 15, 20, 25, 30, 60, 120 and 180 minutes, respectively, 2 ml of the resulting "dispersions" were taken in each case. A loop volume of 100 µl was used from this sample volume for HPLC analysis (repeated test). In a second series, the tablet was placed in the release vessel containing artificial intestinal juice and measurements were made as described above after 1, 2, 3, 4, 5 and 6 hours. In both investigations, the fluid volume removed was not replen-

**Table 1.** Octanol-water distribution of harpagoside and harpagoside in *Harpagophytum* extract for differing pH values and a temperature of 20 °C or 37 °C (repeated tests).

| | Harpagoside | | Extract | |
|---|---|---|---|---|
| pH | 20 °C | 37 °C | 20 °C | 37 °C |
| 2 | 3.4 | 3.7 | 3.1 | 3.7 |
| 3 | 3.3 | 3.9 | 3.3 | 3.9 |
| 4 | 3.4 | 3.6 | 3.2 | 3.8 |
| 5 | 3.5 | 3.9 | 3.3 | 3.7 |
| 6 | 3.3 | 3.8 | 3.5 | 4.0 |
| 7 | 3.3 | 4.0 | 3.3 | 3.7 |
| 8 | 3.7 | 4.1 | 3.5 | 4.0 |
| 9 | 3.5 | 4.1 | 3.7 | 4.2 |

* Schwabe GmbH, Karlsruhe.

**Table 2.** Harpagoside release from tablets batch 9102 in artificial gastric fluid and subsequent degradation (mean value of 8 tablets, SD standard deviation).

| | Sampling Times [min.] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time [min.] | 5 | 10 | 15 | 20 | 25 | 30 | 60 | 120 | 180 |
| A (mg) | 0.8 | 4.4 | 7.9 | 11.8 | 15.8 | 17.4 | 17.2 | 16.6 | 15.9 |
| (%) | 0.2 | 1.2 | 2.1 | 3.2 | 4.3 | 4.7 | 4.7 | 4.5 | 4.3 |
| B (mg) | 2.1 | 7.2 | 11.8 | 14.6 | 17.1 | 17.8 | 17.6 | 16.8 | 15.6 |
| (%) | 0.6 | 2.0 | 3.2 | 4.0 | 4.7 | 4.9 | 4.8 | 4.6 | 4.3 |
| C (mg) | 0.9 | 6.6 | 10.0 | 13.4 | 15.7 | 17.3 | 17.1 | 16.7 | 16.3 |
| (%) | 0.3 | 1.6 | 2.8 | 3.7 | 4.4 | 4.8 | 4.8 | 4.7 | 4.6 |
| D (mg) | 0.7 | 5.1 | 10.8 | 14.9 | 17.1 | 17.4 | 17.3 | 16.5 | 16.2 |
| (%) | 0.2 | 1.4 | 2.9 | 4.1 | 4.7 | 4.7 | 4.7 | 4.5 | 4.4 |
| E (mg) | 1.4 | 4.7 | 7.7 | 10.6 | 12.8 | 15.3 | 16.9 | 16.3 | 15.7 |
| (%) | 0.4 | 1.3 | 2.1 | 2.9 | 3.5 | 4.2 | 4.7 | 4.5 | 4.4 |
| F (mg) | 1.8 | 6.6 | 12.0 | 16.6 | 17.0 | 17.0 | 16.9 | 16.0 | 15.2 |
| (%) | 0.5 | 1.8 | 3.3 | 4.6 | 4.7 | 4.7 | 4.7 | 4.4 | 4.2 |
| G (mg) | 1.5 | 6.6 | 10.6 | 12.0 | 14.2 | 16.9 | 16.8 | 16.3 | 16.1 |
| (%) | 0.4 | 1.8 | 2.9 | 3.3 | 3.9 | 4.6 | 4.6 | 4.5 | 4.4 |
| H (mg) | 0.7 | 4.7 | 9.2 | 13.3 | 16.4 | 17.3 | 17.3 | 16.6 | 16.1 |
| (%) | 0.2 | 1.3 | 2.5 | 3.6 | 4.5 | 4.7 | 4.7 | 4.5 | 4.4 |
| Mean [mg] | 1.3 | 5.6 | 10.0 | 13.4 | 15.8 | 17.0 | 17.1 | 16.5 | 15.9 |
| SD | 0.5 | 1.1 | 1.6 | 2.0 | 1.6 | 0.8 | 0.2 | 0.3 | 0.4 |

ished. Furthermore, the harpagoside content was analysed immediately after solving 200 mg Harpagophytum extract WS 1531 in 900 ml artificial gastric fluid (n = 8) or intestinal fluid (n = 8).

| | |
|---|---|
| Apparatus: | Rotating paddle device, Erweka<br>Speed: 90 rpm |
| Test temperature: | 37 °C |
| Test fluid: | artificial gastric fluid* or intestinal fluid** |
| Initial test fluid volume: | 900 ml |
| Assay: | HPLC |
| Sample volume: | 2 ml |
| Injection volume HPLC: | 100 µl |

* Artificial gastric juice prepared in accordance with USP XXII using the enzyme pepsin: 2 g NaCl and 3.2 g pepsin in 7 ml 0.085 m HCl, filled with water to 1,000 ml (pH 1.2)

** Artificial intestinal juice prepared in accordance with USP using the enzyme pancreatin: 6.8 g potassium phosphate in 250 ml water plus 190 ml 0.2 n NaOH plus 400 ml water. After adding 10.0 g pancreatin (adjusted to a pH of 7.5 ± 1 using 0.2 n NaOH) filled with water to 1,000 ml.

## Results

Both, pure harpagoside and harpagoside fom *Harpagophytum* extract have an octanol-water distribution coefficient of about 4. The influence of temperature and pH is presented in Table 1.

The assay for batch 9102 had an intra-assay coefficient of variation of 0.5% (mean value 3.391 µg/loop, SD 0.017), an inter-assay coefficient of variation of 1.3% (mean value 3.388 µg/loop, SD 0.043). The recovery for 10 mg was 98 –105%, for 15 mg 96–103% and for 20 mg 94–102%. The mean harpagoside content of batch 9102 tablets was 16.42 mg (S.D. 0.2). When related to the extract content of 200 mg, this corresponds to a extract harpagoside content of 8.2% (S.D. 0.1). When related to a tablet weight of 365 mg (100%), this corresponds to a harpagoside content of 4.5% (S.D. 0.049).

**Table 3.** Harpagoside release from tablets batch 9102 in artificial intestinal fluid and its influence over a 6 hour period (N = 8 tablets).

| | Sampling Times [h] | | | | | |
|---|---|---|---|---|---|---|
| Tablet | 1 | 2 | 3 | 4 | 5 | 6 |
| A | 17.6 | 17.3 | 15.4 | 17.7 | 17.7 | 17.7 |
| B | 17.3 | 17.5 | 16.9 | 17.1 | 17.4 | 17.4 |
| C | 17.7 | 17.3 | 17.6 | 17.6 | 17.5 | 17.4 |
| D | 16.9 | 17.5 | 16.5 | 17.5 | 17.4 | 17.3 |
| E | 17.1 | 17.1 | 17.2 | 17.2 | 17.2 | 17.3 |
| F | 17.2 | 17.4 | 17.6 | 17.3 | 17.5 | 17.6 |
| G | 15.5 | 15.5 | 15.9 | 15.5 | 17.4 | 15.7 |
| H | 15.4 | 17.7 | 17.7 | 17.4 | 15.8 | 17.6 |
| MEAN | 16.8 | 17.2 | 16.8 | 17.2 | 17.3 | 17.3 |
| SD | 0.8 | 0.7 | 0.8 | 0.7 | 0.6 | 0.6 |

On average the tablets disintegrated after 18 ± 3 minutes (mean ± SD; range 13 min 5 sec to 22 min 25 sec). The white coating disintegrated very uniformly (blistering off) within 6 minutes, whereas the extract core showed some irregularity during disintegration, but fully disintegrated on average after further 12 minutes.

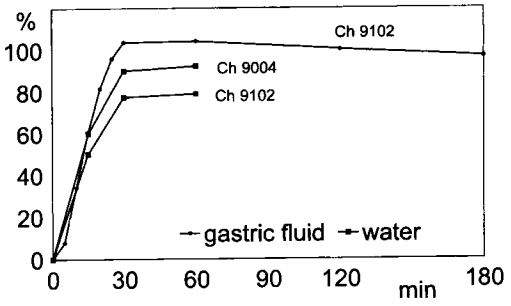


**Fig. 2.** Mean harpagoside release [%] from *Harpagophytum* tablets related to a mean harpagoside quantity of 16.5 mg per tablet

The tablets taken from Batch 9102 release in the paddel model the principal substance harpagoside with a mean half life of 13.5 min., a 90% release time of 23 minutes and a 95% release time of 25 min. (Table 2). Release in the paddle model in water and with 100 rpm resulted in a 20% lower harpagoside release (personal communication, Fa. Dr. W. Schwabe) (Fig. 2). Harpagoside degradation in artificial gastric and intestinal juice for a period of 3 and 6 hours, respectively, is presented in Tables 2 and 3.

## Discussion

Neutral, uncharged organic substances always disperse in a substance-specific ratio between phases when in contact with an aqueous phase and an organic solvent insoluble in water (lipophilic phase). Knowledge about the physicochemical properties of compounds in-vitro allows us to draw some reasonable conclusions about the behavior of drugs in vivo during absorption and the possible distribution of the active ingredients in body fat. Clinical differences in substances with various octanol water distribution coefficients are seen within a range of 3 orders of magnitude (Chrubasik et al., 1993). Our results indicate that the relatively hydrophilic component harpagoside will not accumulate in fat tissue as supposed from a study comparing the pharmacokinetics of a lipophilic and a hydrophilic substance (Chrubasik et al., 1994). Furthermore, the results from Table 2 indicate rapid release of harpagoside from the tablets within half an hour and a 20% degradation of harpagoside in acid milieu over the following 2.5 hours. This may be bypassed by using enteric coated tablets since harpagoside remains unaffected by the artificial intestinal fluid. The average harpagoside values determined in the assay employed demonstrate that the *Harpagophytum* tablets tested fulfill the DAB requirements concerning the release of active ingredients in production batches. The tablet characterization complies with the requirements of DAB in relation to uniformity of mass and active component content by being aware that harpagoside is only one active principle of the multicomponent extract. Regarding harpagoside, the requirements of the disintegration for quick-release tablets are maintained.

Theoretically, the distribution of harpagoside from *Harpagophytum* extract (a multicomponent system) may differ from that of pure harpagoside due to the content of salts etc., our results, however, show that in vitro there is no evidence for a different distribution ratio under the two conditions (Table 1). Although the tests involving the release of an active ingredient can also be carried out in an aqueous environment, it has been recommended that physiological conditions should be imitated as closely as possible in order to arrive at more objective results (Siewert 1995). There was a 20% difference.

Since the release of harpagoside from tablets is reliable and since extract with a higher harpagoside content is clinically more effective (Chrubasik et al., 1999), it is necessary to know the harpagoside content of medications that varied in Germany in 1996 by two orders of magnitude (Chrubasik et al., 1996c) to be able to maximize treatment success. Declaration of the harpagoside content per tablet on the lable of pharmaceutical preparations is, thus, required.

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