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# Fast HPLC for quality control of *Harpagophytum procumbens* by using a monolithic silica column: method transfer from conventional particle-based silica column☆

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**Abstract**

The applicability of a monolithic C18-bonded silica column for the rapid HPLC separation of ingredients in medicinal plants and their phytopharmaceutical preparations has been evaluated in the author's laboratory. In this presentation, an existing method for the determination of the iridoid glycoside harpagoside in *Harpagophytum procumbens* (Devil's Claw) was successfully transferred from a conventional particle-based C18 silica column to a monolithic silica column. The very high porosity of the stationary phase allows chromatography with a much lower backpressure than on conventional columns. Therefore, the flow rate could be easily increased from 0.8 mL/min (particle-based column) to 5 mL/min (monolithic column) and the run-time reduced from 30 to 5 min (that is a reduction about 85%!), without losing any chromatographic resolution of the compound of interest. The amount of harpagoside was measured with the original method on a conventional particle-based silica column and on the adapted method on a monolithic silica column. The statistical mean *t*-test showed no significant differences of the variances and the means indicating that the fast HPLC method is an acceptable alternative. The shorter analysis time makes the method very valuable for commercial quality control of *Harpagophytum* extracts and its pharmaceutical preparations.


*Keywords:* HPLC; Monolithic silica column; Method transfer and method validation; *Harpagophytum procumbens* (Devil's Claw)

## 1. Introduction

In recent years, monolithic stationary phases have attracted considerable attention in high performance liquid chromatography due to its simple preparation procedure, unique properties and excellent performance [1–3].

The monolithic C18 silica column differs from classical silica columns since it consists of a silica rod, instead of particles. The very high porosity of the stationary phase allows chromatography with much lower back-pressure than on conventional columns, and therefore, flow rates up to 9 mL/min become possible.

Monolithic silica columns are being more and more commonly used and several applications have already been published [4–7]. However, an evaluation of the applicability of such phases to the analysis of medicinal plants has been little reported in the literature [8–10].

In our laboratory, many separations for medicinal plants and their commercial products have been developed on different types of conventional HPLC columns with particle-based silica packings [11–13]. Because of these complex matrices, run-times of 30–60 min are not uncommon and it takes over-night sample runs to get the results needed for quality control of batches. Therefore, it was interesting to investigate whether these separations could be transferred to C18

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monolithic columns to operate at much higher flow rates that shorten run-times.

In this study, an established high performance liquid chromatography (HPLC) separation method for the extract of *Harpagophytum procumbens* (Devil's Claw), developed on a conventional, particle-based silica column, was transferred to a monolithic column and validated in compliance with ICH guidelines [14].

*H. procumbens* is a plant found in different parts of southern and southwestern Africa (Kalahari desert, Namibia steppes). The common name of Devil's Claw is derived from the herb's unusual fruits, which seem to be covered with numerous small hooks. Phytopharmaceutical preparations of the dried hydroalcoholic or aqueous extracts of the tubular secondary roots of *H. procumbens* are recommended by an ESCOP monograph and have been successfully used in treatment of degenerative rheumatism and painful arthrosis as well as for loss of appetite and dyspeptic complains [15,16]. An improvement of mobility and a reduction of pain have been observed in many clinical trials with extracts of the secondary tubers of Devil's Claw [17,18].

Ten compounds have been found in *H. procumbens* roots: the iridoid glycosides harpagoside and 8-*p*-coumaroyl harpagide, the phenylethanoid glycoside acteoside, and cinnamic acid are some of them (for chemical structures, see Fig. 1) [19]. The major pharmacological active ingredient is harpagoside, and related iridoid glycosides, which occur in smaller amounts. Therefore, harpagoside is used as a marker compound to assess on the quality of *H. procumbens*.

## 2. Experimental

### 2.1. Chemicals

Solvent A was prepared by adding *o*-phosphoric acid (Suprapur, Merck, Darmstadt, Germany) to water, purified by a Milli-Q water purification system (Millipore, Eschborn, Germany), up to pH 2.0. Solvent B was acetonitrile and of HPLC-grade (LiChrosolv, Merck, Darmstadt, Germany).

### 2.2. Standards

The reference standards of harpagoside, and acteoside were purchased from Roth (Karlsruhe, Germany), 8-*p*-coumaroyl harpagide was from Addipharma (now: Phytolab, Hamburg, Germany) and cinnamic acid was from Merck.

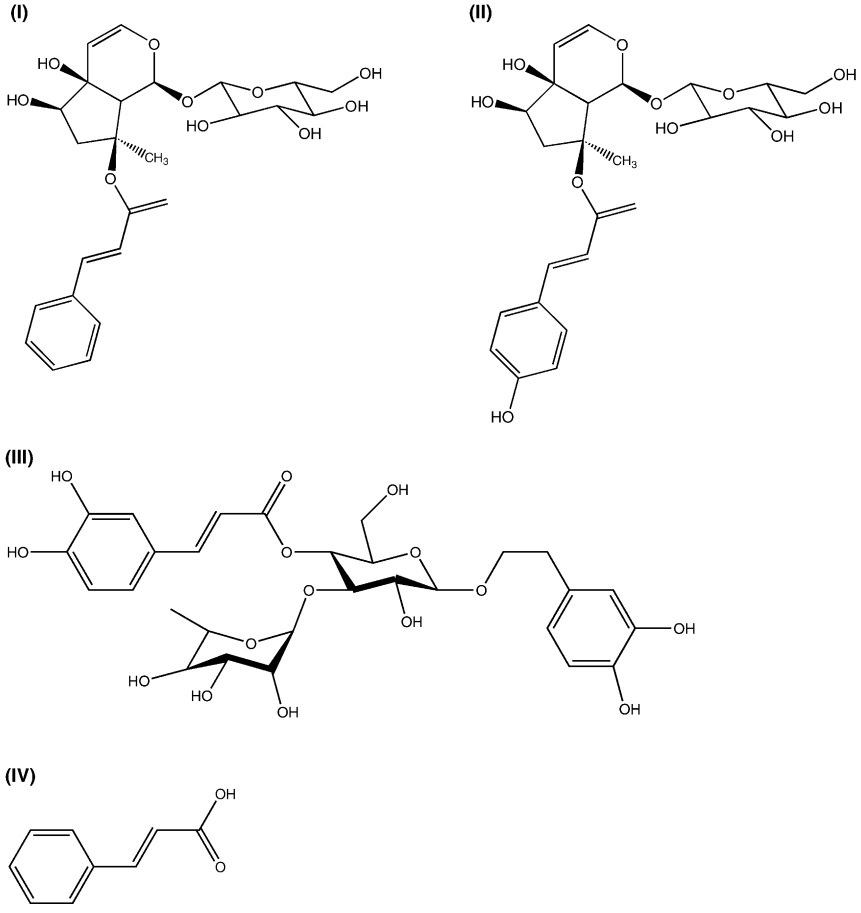


Fig. 1. Chemical structures of the iridoid glycosides harpagoside (I); 8-*p*-coumaroylharpagide (II); the phenylethanoid glycoside acteoside (III) and cinnamic acid (IV).

Quantitative determination was carried out using the internal standard method [20]. One hundred and thirty milligrams of the internal standard (IS) cinnamic acid methylester (Roth) was accurately weighed into a 100-mL volumetric flask and dissolved in methanol. The internal standard solution was found to be stable over the investigated period of 4 weeks at 4 °C.

### 2.3. Equipment

Chromatographic analysis was performed using a Waters Alliance HPLC system (Eschborn, Germany) equipped with 2695XE separation module with degasser, temperature-controlled sample compartment and column heater, and a 2996 photodiode-array detector. Chromatograms were monitored at a wavelength of 278 nm. UV-spectra were taken in the range of 210–440 nm.

For system control, data acquisition and data processing the Millennium$^{32}$ V.4.0 client/server software (Waters, Eschborn, Germany) was used.

Statistical analysis was calculated using the MVA (Novia, Saarbrücken, Germany) statistical software (Version 1.0).

### 2.4. Plant material

The dried hydroalcoholic extract of Devil's Claw (SteiHap69, Steiner and Co., Berlin, Germany) used in this study was produced from the dried secondary root tubers of *H. procumbens* using ethanol 60% by an appropriate procedure. The weight ratio drug/extract is 4.4–5.0:1.

### 2.5. Sample preparation

Two hundred and fifteen milligrams of *H. procumbens* extract were exactly weighed into a 25-mL volumetric flask and extracted with 20 mL methanol by use of a ultrasonic bath (Sonorex RK1029, Bandelin, Berlin, Germany) for 20 min at room temperature. One millilitre of the internal standard solution was added and the mixture was made up to volume with methanol. An aliquot of the preparation was filtered through a 0.45-μm-PTFE membrane filtration cartridge (Gelman Sciences, Dreieich, Germany) directly into a vial and transferred into the HPLC autosampler.

Powdered samples of the phytopharmaceutical dosage form (Sogoon tablets, Steiner and Co., Berlin, Germany), equivalent to about 215 mg of the extract, were treated in the same way.

### 2.6. Chromatographic conditions

#### 2.6.1. Method on conventional particle-based column

The conventional silica column used for chromatography was a Hypersil ODS, 5-μm particle size and a dimension of 125 mm × 4 mm (Phenomenex, Aschaffenburg, Germany).

A linear gradient was started with 1% solvent B and was increased within 16 min to 50% B. After an isocratic elution with 50% B for 1 min, the gradient was returned to the initial conditions within 3 min to re-equilibrate the column. The flow rate was 0.8 mL/min and the column temperature was 30 °C.

#### 2.6.2. Method on monolithic column

The monolithic column was a Chromolith Performance RP-18e with the dimension 100 mm × 4.6 mm (Merck, Darmstadt, Germany). Two 100 mm columns were coupled in series. A linear gradient was started with 1% solvent B and was increased within 2 min to 50% B. After an isocratic elution with 50% B for 1 min, the gradient was returned to the initial conditions within 1 min to re-equilibrate the column. The flow rate was 5.0 mL/min and the column temperature was 30 °C.

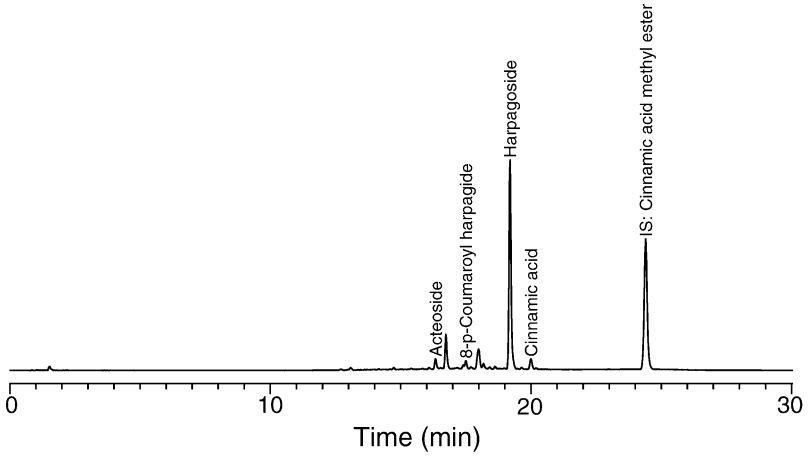


Fig. 2. Chromatogram at 278 nm of the iridoid glycoside harpagoside and other compounds in *Harpagophytum procumbens* on Hypersil ODS. Conditions: see Section 2.

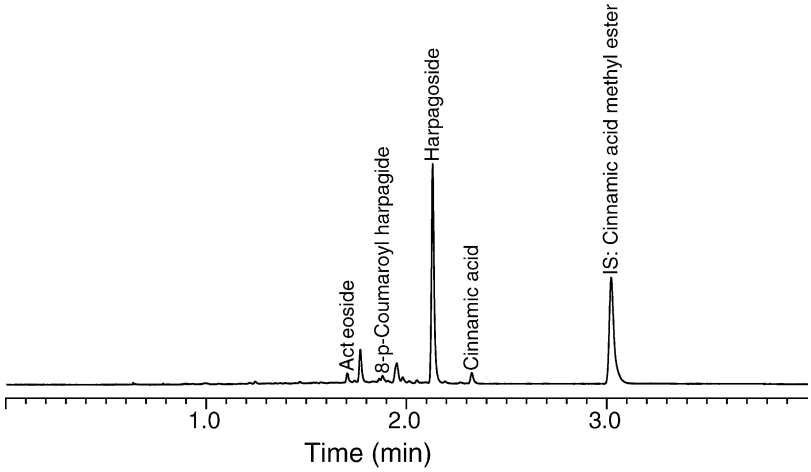


Fig. 3. Chromatogram at 278 nm of the iridoid glycoside harpagoside and other compounds in *Harpagophytum procumbens* on Chromolith Performance RP-18e. Conditions: see Section 2.

## 3. Results and discussion

Quantification of the main constituent of *H. procumbens* has been already established using the HPLC method on a conventional particle-based column. The method allowed good separation and quantification of the main constituent within 30 min. The marker compound harpagoside eluted at 19.2 min and the IS (cinnamic acid methylester) at 24.4 min. A typical chromatogram is shown in Fig. 2.

By using a monolithic column the flow rate could easily be increased to 5 mL/min. By decreasing the gradient time proportionally, a 5-min run-time was achieved with an elution of harpagoside at 2.1 min and the IS at 3.0 min. Surprisingly no loss of resolution for the main compounds was discovered (Fig. 3) if two columns (each with a dimension of 100 mm × 4.6 mm) are coupled in series.

The use of monolithic silica columns at high flow rates required a fast data acquisition to have at least 20 data points per peak. Therefore, the data acquisition rate (or detector sampling rate) was increased from 1 (conventional particle-based columns) to 10 data points per second (monolithic columns). The peaks of interest in the chromatogram were identified by comparison of the retention times and the UV-spectra of the peaks with those from the reference standards.

The amounts of harpagoside were measured with the IS cinnamic acid methylester for the original method on a conventional particle-based silica column and for the transferred method on a monolithic silica column. Values given for harpagoside were calculated using a correction factor of 7.622 in accordance with published data [20].

The new method has been validated in compliance with ICH guidelines and compared with the old method. The

Table 1
Comparison of the determination of harpagoside in *Harpagophytum procumbens* extract and a commercial product using the HPLC method on a conventional particle-based column and a monolithic column

| | Conventional column | Monolithic column |
|---|---|---|
| *Harpagophytum procumbens* extract (SteiHap69) | | |
| Number of values | 6 | 6 |
| Mean (mg harpagoside/100 mg) | 2.006 | 2.017 |
| 95% confidence interval (mg harpagoside/100 mg) | 1.982–2.031 | 1.992–2.042 |
| R.S.D. (%) | 1.01 | 1.16 |
| Mean *t*-test | | |
| *F*-test (5, 5, 95%) | No significant differences of the variances | |
| *t*-test (10, 95%) | No significant differences of the means | |
| Commercial product (Sogoon tablets) | | |
| Number of values | 6 | 6 |
| Mean (mg harpagoside/tablet) | 13.019 | 13.075 |
| 95% confidence interval (mg harpagoside/tablet) | 12.984–13.054 | 13.040–13.111 |
| R.S.D. (%) | 0.56 | 0.26 |
| Mean *t*-test | | |
| *F*-test (5, 5, 95%) | No significant differences of the variances | |
| *t*-test (10, 95%) | No significant differences of the means | |

comparison of the methods was determined by assaying six replicates of the same sample. Each replicate was injected three times for each method. The statistical mean *t*-test showed no significant differences of the variances and the means for both methods (see Table 1).

## 4. Conclusion

This study has shown a very easy and successful transfer for a HPLC separation method for *H. procumbens*, developed on a conventional particle-based silica column to a monolithic silica column. The monolithic column "Chromolith" was found to give comparable results to a conventional 5 μm particle-based column with the advantage, that a higher flow rate could be used due to a flat van Deemter curve. So the flow rate was increased from 0.8 up to 5 mL/min without any loss of resolution. The total run-time was reduced from 30 to 5 min, this is a six-fold increase of the throughput from the separation on the conventional column! Even if you compare the costs of the columns—two Chromolith-columns in series are three times as expensive as one Hypersil-column—the new method is very valuable for commercial quality control where speed of analysis is crucial to free up HPLC systems and analyst time for other projects.

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