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Development and validation of a new HPLC-UV method for the determination of harpagoside in creams containing Devil's claw (*Harpagophytym procumbens* DC. ex Meisnn)

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Abstract: Harpagoside, an iridoid glycoside, is the primary active compound found in *Harpagophytum procumbens* DC. ex Meisn. The main goal of this work was to develop a high-performance liquid chromatographic (HPLC) method for measuring the amount of harpagoside in different cream samples that is rapid, straightforward, and accurate. Prior to chromatographic separation, liquid-liquid extraction procedure was employed for the preparation of samples, which is now the predominant extraction technique due to its simplicity, speed, and efficiency. Reversed phase C18 (5 µm × 4.6 mm × 150 mm) analytical column, methanol and water (60:40 v/v) at a flow rate of 1.1 mL/min, and a UV detector that detected at 272±1 nm were used to achieve the chromatographic separation. The linear behaviour of the proposed approach was examined in the 0.1-30 ng/mL range (r^2=0.9998). The proposed method is in alignment with the criteria that is established by International Conference on Harmonisation (ICH) about the accuracy, precision, repeatability, specificity, robustness and detection and quantification. Limit of Detection and Limit of Quantification are determined to be 0.03 and 0.1 ng/mL whereas relative standard deviation was determined to be less than 3.42% for daily and hourly measurements. The suggested analytical process is a productive way to measure and routinely analyse harpagoside in creams containing Devil's claw.

Keywords*:* Harpagoside; Devil's claw; HPLC-UV; method validation. © 2025 ACG Publications. All rights reserved.

1. Introduction

Harpagophytum procumbens DC. ex Meisn. (Pedaliaceae), an indigenous plant of the Kalahari Desert in Africa, is rare and found exclusively in Southern Africa. It is native to South Africa, Namibia, Botswana, and South-Eastern Africa, where it is traditionally harvested from the wild [1]. It was first introduced to Europe by O.H. Volk in 1953. *H. procumbens* also known as Devil's claw, was added to the French pharmacopoeia in 1989. In southern Africa, it is traditionally used to treat liver, stomach, gallbladder, kidney, and pancreas ailments, as well as to manage fever, pregnancy-related disorders, and bleeding. In Europe, *H. procumbens* is commonly used for metabolic disorders, diabetes, and age-related conditions, though its effectiveness lacks sufficient scientific evidence [2].

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Harpagoside (Figure 1) with the chemical name [(1S,4aS,5R,7S,7aS)-4a,5-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6(hydroxymethyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopenta[c]pyran-7-yl](E)-3-phenylprop-2-enoate. Harpagoside, a prominent iridoid glycoside derived from *H. procumbens*, is extensively investigated for its pharmacological potential, particularly in the context of inflammatory and neuropathic disorders. Due to its significant anti-inflammatory and neuroprotective effects, it is commonly employed as a phytochemical marker in standardized preparations aimed at the management of lower back and joint pain [3].

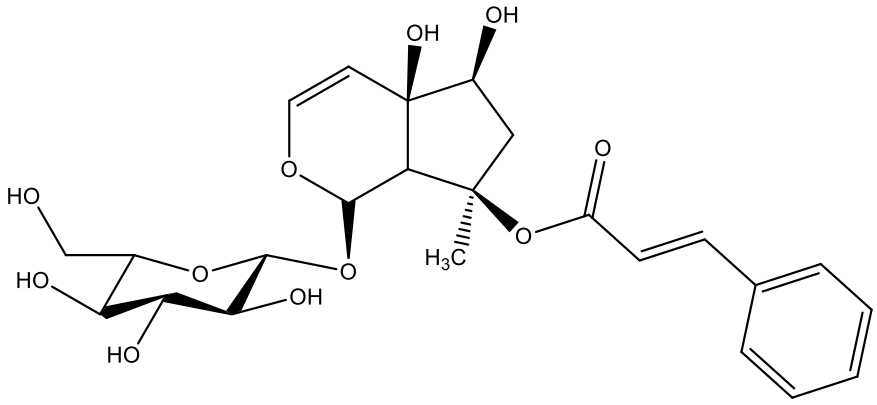


Figure 1. Chemical structure of harpagoside

Literature reports indicate that harpagoside levels can be effectively determined using HPLC across various sample types, including botanical preparations, medicinal products, and plasma specimens [4-9]. A significant drawback of conventional analytical approaches lies in their dependence on high-cost instrumentation, intricate sample preparation workflows, and suboptimal sensitivity, which collectively hinder their broader applicability. Although Devil's Claw is widely preferred by healthcare professionals, patients and the public, there is no method for the determination of harpagoside in cream samples. The advantage of the analytical method we developed over existing analysis methods is that it provides an easy sample preparation procedure, high sensitivity and enables the analysis of the active ingredient in cream samples with complex sample matrices.

The aim of the study is to establish and validate the HPLC technique that helps us understand harpagoside in creams containing Devil's claw without interaction with other products. With this analytical method, harpagoside determination was carried out for the first time quickly and easily without the need for a derivatization step. As for the developed method, the analysis for cream products claimed to contain harpagoside can be done quickly and easily for many laboratories. If validation studies are investigated, this method can be safely used in future routine analyses.

2. Experimental

2.1. Instrumentation and Reagents

For liquid chromatography, LC-20 from the company Shimadzu from Japan was used. LC-20 is upgraded with an SPD-20A HT UV detector which is calibrated to the wavelength of 272 nm, an LC-20 AT pump, a SIL AT-HT autosampler component, and a CTO 10 AC column oven, was readied for the HPLC studies. On a GL Sciences (Japan) C18 (ODS) column with a measurement of 4.6 mm I.D., 150 mm length, and 5 µm particle size, chromatographic separation was done isocratically at 30 ºC. With flow rate of 1.1 mL/min, the mobile phase consisted of methanol-water containing 1 mL/L triethylamine.

The study's entire chemical supply was of analytical reagent quality. Sigma Aldrich, St. Louis, Missouri, USA, facilitated the acquisition of harpagoside. Methanol, and trimethylamine are all HPLC grade and are sourced from Merck (Darmstadt, Germany). The water that was used was cleaned using an ultrawater filtration technology from Water Human (Japan).

2.2. Methods

A stock solution of harpagoside (1 mg/mL) was made using an 60:40 methanol:water combination. It was necessary to reduce the concentration of the stock solution with methanol in order to create the working standard solutions in various concentrations prior to experiments. For HPLC analysis, working standard solutions were aliquoted in 20 µL quantities. The chromatograms were analysed using the peak areas against the concentration of the harpagoside components.

2.3. Preparation of the Calibration Curve

An analysis of harpagoside working standard solutions at various concentrations was done to create the calibration curve. A linear least-squares regression analysis was used to estimate the linear concentration ranges of the approach for both substances. The calibration curve equation was derived using the formula $y = ax+b$, where x denotes the drug ingredients' concentrations in ng/mL and y denotes the peak regions. Each level of concentration was examined on five separate occasions.

2.4. Uncertainty Assessment

The main sources of uncertainty were evaluated, including contributions from the compound's purity (ustandard), weighing process (uweighing), calibration curve parameters (ucalibration), recovery (urecovery), and repeatability (urepeatability). The combined uncertainty (ucombined) was calculated using the following formula:

$$u_{Combined} = \sqrt{(u_{standard})^2 + \left(u_{weighing}\right)^2 + \left(u_{recovery}\right)^2 + (u_{curve})^2}$$

The overall expanded uncertainty (uexpanded) was determined at a 95% confidence level by multiplying the combined uncertainty by a coverage factor (k) of 2. The uncertainty calculations were performed in accordance with the EURACHEM Guide [10] and relevant literature [11].

2.5. Extraction Process for Application the Method to Devil's Claw Products

To extract harpagoside from creams items, a number of methods of extraction have been developed, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). The liquid-liquid extraction (LLE) methodology was first explored using a range of extraction solvents, mixtures, and quantities of extraction solvents in order to create an efficient method of extracting harpagoside from creams. In order to optimize liquid-liquid extraction, acetonitrile, methanol, dimethyl sulfoxide, chroloform and ethanol solvents were tested alone as well as their solvent mixtures with water. Employing the solid phase extraction (SPE) technique, experiments were conducted using various elution procedures employing reversed phase and normal phase sorbents (C18-N, C8, NH2, C18 Resprep cartridges; 6 mL, 1.000 mg). In LLE, the highest recovery was observed in the methanol-water solvent mixture. The recovery values for the methanol-water solvent mixture varied between 80.16% and 90.88%. However, the highest recovery value among the sorbents tested for SPE was found to be 72.45%. *Following the results of these studies, which demonstrated greater recover values and increased efficiency from LLE, the following procedure was chosen to be used. A 1:1 mixture of methanol and water (1 mL) was added to an Eppendorf tube containing 0.1 g of the cream sample. The mixture was vortexed for 30 seconds, followed by centrifugation at 4000 rpm for 10 minutes. After centrifugation, 800 µL of the supernatant was filtered through a 0.45 µm polyethersulfone filter (Dainippon Seiki, Kyoto, Japan) and transferred to a 1.5 mL HPLC vial. For each duplicate, 20 µL of the sample was injected for HPLC analysis.

2.6. Cream Materials and Ingredients

All cream samples analyzed were obtained commercially. All creams containing Devil's claw are in the from of an oil-in-water emulsion. It contains solid or liquid form of Devil's claw extract as the active ingredient. The water phase consisted of water, glycerin and urea. The oil phase consisted of vegetable oils and stearyl alcohol. It usually contained sodium hydroxide as a thickener and essesntial oils that provide a cooling sensation as excipients.

3. Results and Discussion

3.1. Method Development

Reverse-phase HPLC was the method of choice for figuring out how much harpagoside was included in Devil's claw creams. Using different kinds of columns at varying temperatures, preliminary tests were done to ascertain the optimal chromatographic conditions. At 30 ºC, a higher resolution value (more symmetrical and sharper peaks) was obtained using a C18 column with the following dimensions: 4.6 mm I.D., 150 mm length, and 5 µm particle size. The chromatographic separation was carried out by isocratic elution at room temperature on a GL Sciences (Japan) C18 (ODS) column with methanol and water (60:40, v/v) containing with a flow rate of 1.1 mL min-1. The maximum absorption of harpagoside was measured by UV spectrophotometer at 272 nm. Under these circumstances, the retention period of harpagoside is 2.52 ± 0.01. The ideal conditions were determined by measuring peak areas and resolution values. Figure 2 (a-c) displays a good representation for chromatograms of the cream sample, standard solution, and blank solution. Table 1 displays the chromatographic separation process quality and the system appropriateness characteristics of the approach.

Table 1. Chromatographic system suitability parameters

Capacity factor*	Resolution*	HETP*	Tailing factor*	Asymmetry factor*
6.88	1.22	0.11	0.93	0.71

*The average parameter values for every point in the calibration analysis are presented.

3.2. Method Validation

The following International Conference on Harmonisation (ICH) standards were adhered to approach for validation [12]. EURACHEM/CITAC guidelines and previous studies were used to assess and quantify the resources [13-16].

3.2.1. Preparation of the Calibration Curve

The calibration curve was created through the examination of standard harpagoside solutions ranging in concentration from 0.1 to 30 ng/mL. The method's linear concentration ranges (each concentration was examined as five replicates) were identified using linear least-squares analysis. The calibration curve's equation, $y = 10213x + 924.72$, where y represents the peak areas, was computed and where x represents the harpagoside concentrations in 0.1–30 ng/mL.

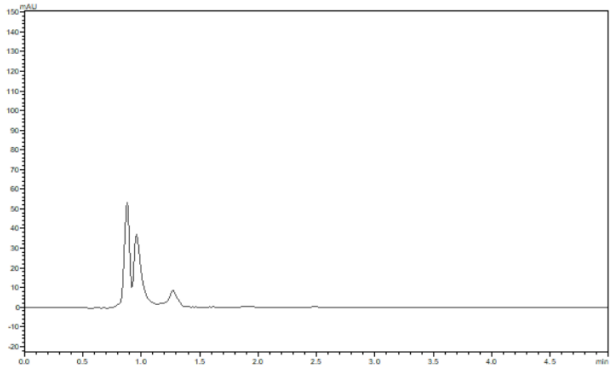
3.2.2. Limit of Dedection (LOD) and Limit of Quantitation (LOQ) Values of the Method

The following formula was used to calculate: LOD or LOQ=kSDa/b, where k=3 for LOD and 10 for LOQ, SDa being the intercept's standard deviation, and b being the slope. Table 2 provides an overview of the characteristics governing the analytical performance of the suggested approach.

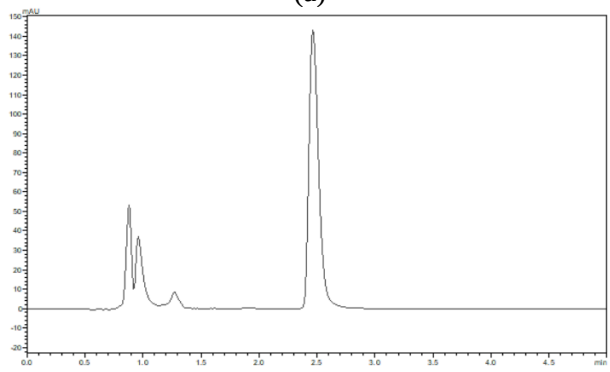
Table 2. Method's analytical parameters

Parameters	Method
Linearity range* (ng/mL)	0.1-30
Regression equation	y=10213× + 924.72
Slope±SD	10213±81
Intercept±SD	924.72±13.44
Correlation coefficient, r^2	0.9998
LOD (ng/mL)	0.03
LOQ (ng/mL)	0.1

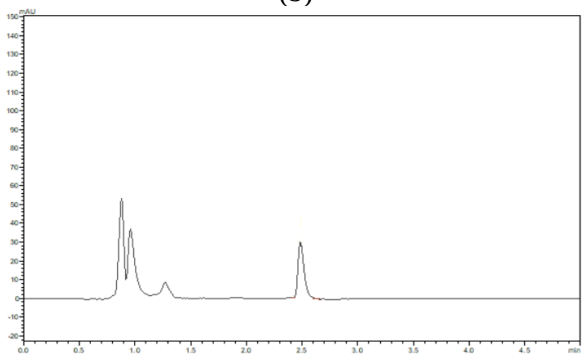
*n=5 matches the replicated analysis for every stage.



(a)



(b)



(c)

Figure 1. Blank (aqueous medium) **(a),** standard solution (10 ng/mL standard harpagoside solution) **(b),** real sample (cream sample) **(c)**

3.2.3. Accuracy, Precision and Recovery

QC samples at three concentration levels were determined in order to evaluate accuracy and precision. Devil's claw cream samples were made using QC samples at three different concentrations (0.1, 5.0, and 30.0 ng/mL), which may be categorized as low, medium, and high concentration (n=5). Recovery values were used to indicate accuracy, and RSD values of the recovery findings in five replicate tests were used to describe precision in the recovery research. Through the extraction of spiked cream samples and comparison with peak areas obtained from the identical amounts of liquid unextracted harpagoside solutions, the absolute recovery of harpagoside from Devil's claw cream samples was investigated. By comparing the quantities added to spiked and measured by the calibration curve, the mean relative recovery was determined to be 99.50%.

For daily precision and accuracy, five duplicate samples at different level of concentrations were examined within the same day; for hourly precision and accuracy, the samples were assayed five days apart. Every daily and hourly assay's RSD value was less than 3.42%. Table 3 summarizes all of these results and shows that good precision and accuracy were noted. The recovery percentages of 97.64, 98.20, and 97.97 are like those found in our earlier research [17].

Table 3. Accuracy and precision of the method

Existant concentration (ng/mL)	**Added concentration (ng/mL)**	**Found concentration (ng/mL) (Mean±SD)**	**Recovery (%)**	**RSD of intraday variation**	**RSD of interday variation**
5	0.1	4.98±0.03	97.64	1.66	3.10
5	5	9.82±0.05	98.20	1.83	3.42
5	30	34.29±0.11	97.97	1.70	3.25

*For each concentration n=5

3.2.4. Stability

Spiked harpagoside Devil's claw cream standards at 1.0, 5.0, and 30 ng/mL were used to investigate the effects of freezing and thawing harpagoside concentrations. The standards underwent four freeze-thaw cycles prior to analysis. Additionally, the stability of harpagoside in spiked Devil's claw creams was assessed after being kept at room temperature for 24 hours and at 20 ºC for 21 days. Harpagoside stock solutions kept at -20 ºC were stable for at least 30 days. There was no discernible drop in the harpagoside concentration of Devil's claw creams after 30 days.

3.2.5. Robustness

Determining the QC samples at three concentration levels as mentioned in the validation section above was how robustness was evaluated (n=3). To assess the method's robustness, several parameters are adjusted, including the mobile phase's liquid phase contents, temperature in the column oven, flow rate, and methanol concentration. The ratio of the mobile phase (methanol-water) was adjusted from 60:40 to 65:35 and 55:45. Additionally, the flow rate was adjusted from 1.1 to 1.0 and 0.9 mL/min, while the column temperature was adjusted from 30 ºC to 25 ºC and 35 ºC. Peak area and resolution were not significantly impacted by these modifications. Table 4 illustrates the robustness of the technique with low RSD values.

Table 4. Robustness of the method

Condition	Value	Recovery%	RSD%
Flow rate mL/min	1.0	96.18	2.79
	1.2	96.92	2.86
Mobile phase composition	55:45	95.77	2.37
(methanol:water)	65:35	95.56	2.25
Column temperature ºC	25	99.51	0.94
	35	99.37	0.91

*For each concentration n=5

3.3. Assessment of Uncertainty

The uncertainty of the analytical method was evaluated and expressed as a percentage (%) at a 95% confidence level for all analytes in the calculated parameters. The results, summarized in Table 5, indicate that the uncertainty values are within acceptable limits. The contribution of uncertainty due to sample weighing was found to be negligible and, therefore, was not included in the final presentation.

Table 5. Estimation of uncertainty budget of the method (%)

$u_{Standard}$	$u_{Calibration}$	$u_{Recovery}$	$u_{Repeatability}$	$u_{Combined}$	$U_{Expanded}$
0.35	1.31	0.30	2.01	2.45	4.79

k=2 (95% confidence level)

3.4. Extraction Process

The validated HPLC method was applied to analysed harpagoside concentrations in Devil's claw creams and to evaluate its effectiveness in real-cream formulations. The results, summarized in Table 6, demonstrate the method's accuracy, precision, and reproducibility, making it a reliable tool for routine quality control analysis.

Table 6. Creams material concentrations and method reproducibility

Cosmetic product	Recovery	%RSD	Harpagoside (ng/mL)
Sample 1	%82.74	2.72	20.68
Sample 2	%80.16	3.65	18.21
Sample 3	%83.54	2.68	22.41
Sample 4	%84.60	2.48	24.49
Sample 5	%82.31	2.94	24.87
Sample 6	%86.45	2.10	26.71
Sample 7	%90.88	1.48	27.32
Sample 8	%85.38	2.34	25.22
Sample 9	%81.64	3.13	19.94
Sample 10	%83.60	2.52	22.83

*For each concentration n=5

4. Conclusions

The developed HPLC method provides a rapid, user-friendly, and cost-effective solution with a short retention time of 2.52 minutes and an isocratic mobile phase (methanol and water), eliminating the need for complex derivatization. The method demonstrates strong linearity (0.1–30.0 ng/mL, R^2 = 0.9998), high sensitivity (LOD: 0.03 ng/mL, LOQ: 0.10 ng/mL), excellent precision (RSD < 3.42%), and a mean relative recovery of 97.93%, ensuring accuracy and reproducibility. A key advantage is its fast and efficient sample preparation, involving a simple 1:1 methanol-water extraction, centrifugation, and filtration, which significantly reduces analysis time while maintaining robustness. The method we

developed stands out by showing shorter retention time, easy sample preparation procedure and high precision compared to existing methods in the literature. In addition, the method developed for the extraction of harpagoside from cream samples increased the extraction efficiency by preventing possible matrix interferences. This makes the method highly suitable for routine quality control applications in the cosmetic and pharmaceutical industries, providing a reliable and efficient tool for harpagoside quantification.

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