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QUANTIFICATION OF A CRUDE EXTRACT FROM *HEDERA COLCHICA*

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ABSTRACT: Crude extract from the leaves of *Hedera colchica* (C. Koch) C. Koch proved to have an antiulcer activity in pharmacological experiences. Hederacolchisid F (HCF) is being the chemical marker of this plant, a simple and reliable HPLC method is developed for the quantitative evaluation of the extract using this compound. The chromatographic separation was achieved using an Eclipse XDB-Phenyl column C-18 (4.6 x 250 mm; 5 μm). The UV detection is performed at 205 nm. All separations were realized at 20°C. The proposed HPLC method is linear in the range studied (r^2> 0.999) for all the analytes. The method is precise with intra- and inter-day variations of less than 1.03%. Precision, sensitivity and linearity are satisfactory in the range studied. Accuracy 99,8±0,9%. The developed HPLC method can be used for the quality control of crude extract from the leaves of *Hedera colchica*.

INTRODUCTION: Colchis ivy - *Hedera colchica* (C. Koch) C. Koch (**Fig. 1)** is an evergreen plant growing in Georgia (Fam. *Araliaceae*) [1]. In folk medicine the different species of ivies are known as plants possessing anticough, diuretic and bronchospasmolitic activities. Recent pharmacological studies revealed anti-inflammatory, antibacterial, bronchospasmolitic, antimicrobial, antifungal, antihelmintic, memory improvement, antiprotozoal and contraceptive activities of triterpen saponins from *Hedera* species; antiviral and antioxidant activities of ivy's crude extract are also reported [2-6].

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Tbilisi State Medical University's Iovel Kutateladze Institute of Pharmacochemistry is developed a crude extract from the leaves of *Hedera colchica*, as an antiulcer active substance on the base of results obtained by pharmacological experiences [7].

FIG. 1: ***COLCHIS IVY - HEDERA COLCHICA*** **(C. KOCH) C. KOCH**

The main constituents in the crude extract of Colchis ivy are triterpen saponins, with

Hederacolchisid F (HCF), as the predominant substance, including other flavonoids and phenolic acids [8]. Since Hederacolchisid F **(Fig. 2)** was selected as the chemical marker of the active substance. The aim of this study was to develop an analytical procedure for the quantitative estimation of Hederacolchisid F in the crude extract of Colchis ivy.

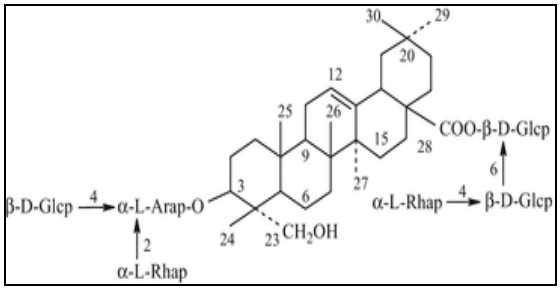

FIG.2: CHEMICAL STRUCTURE FOR HEDERACOLCHISID F

MATERIALS AND METHODS:

The samples of biologically active crude extracts from Colchis ivy, purchased by the TSMU I.K. Institute of Pharmacochemistry (Tbilisi, Georgia) and were protected from light and humidity until required for chemical.

Chemicals and reagents:

HPLC grade acetonitrile of analytical grade were purchased from Merck & Co. Ultrapure water (18 for HPLC analysis was obtained from a Millipore Classic purification system. Hederacolchisid F (HCF) has been isolated from the crude extract of Hedera colchica and the purity determined by HPLC was 99, 0%.

NMR: Structure elucidation was carried out using ^{1}H NMR (Bruker Avance 400MHz), ^{13}C (Bruker Avance 100 MHz).

Chromatographic instrument and conditions: The HPLC system consisted of an Agilent Technologies Model 1100 liquid chromatography, equipped with a vacuum degasser, a quaternary pump, an auto-sampler and a photodiode array detector (DAD) and MS. The system was by Chemstation computer software. The chromatographic separation was achieved using an Eclipse XDB-Phenyl column C-18 (4.6 x 250 mm; 5 μm). Mobile phase was composed of water and acetonitrile (75-25, v/v) with a flow rate of 1ml/min and injection volume of 10μl and all separations were performed at 20°C. The UV spectra were recorded in the detection range of 200-400 nm for all peaks. Quantification was carried out at a single wavelength of 205 nm.

Preparation of standard solution: Standard solution of Hederacolchisid F was prepared in methanol to a final concentration of 1.0 mg/mL. A series of working solutions of Hederacolchisid F (n=5) was prepared in order to obtain various concentration levels (0.002-1.0 mg/mL). The appropriate volume of solution of Hederacolchisid F was introduced in a 10.0 mL volumetric flask and the volume was adjusted to 10.0 mL with mobile phase. All prepared standard solutions were filtered through 0.45 μm membrane filter (Millipore, ref HVPL04700) before HPLC analysis.

Preparation of sample solution: 20.0 mg of the crude extract of Colchis ivy (purchased by the Institute of Pharmacochemistry, Tbilisi, Georgia) was introduced in a 100 mL volumetric flask and solubilised in methanol and the volume was adjusted to 100.0 mL with methanol. Then 2 ml of each solution were filtered through a syringe filter (0.45 mm Millipore) into a HPLC vial.

Validation and assay: The method was validated on the crude extract of Colchis ivy according to the ICH guidelines [8]. The linearity of the HPLC method was performed for Hederacolchisid F. Five different concentrations were prepared and analyzed in triplicate for each concentration. The concentration range was 0.002 to 1.0 mg/mL for Hederacolchisid F. Calibration curves were constructed by plotted peak areas against concentrations. The linearity was assessed by calculating the slope, y-intercept and coefficient of correlation (r^2) using least squares regression. The limits of detection and quantification were considered to be the concentration that produced signal-to-noise ratios of 3:1 and 10:1 respectively.

The precision of the method was evaluated with respect to both intra- and inter-day precision. Intra-day precision was calculated from the analyses of six sample solutions prepared independently on one day. Inter-day precision was evaluated by repeating the same procedure on 2 other days, The standard deviation and the RSD (relative standard deviation)

values were calculated for each day. The accuracy of the method was evaluated using the recovery test. This involved the spiking of known quantities of HCF standard solutions into the real samples. The standard solutions were prepared at three concentration levels (50, 100 and 150%). At each level, samples were analysed in triplicates according to the previously described chromatographic conditions.

RESULTS AND DISCUSSION:

HPLC separation conditions were optimized in order to achieve satisfactory resolution [9]. The optimal separation of the crude extract of Colchis ivy was achieved on the reverse phase column Eclipse XDB-Phenyl C-18 (4.6 x 250 mm; 5 µm). To provide an optimal resolution, the effect of mobile phase was examined. A satisfactory separation was detected with a mobile phase consisting of acetonitrile-water in a ratio of 25/75, v/v. The solution of Hederacolchisid F was prepared in methanol. The retention time of Hederacolchisid F was observed at 10.3 min.

Measurement at 205 nm displayed sufficient sensitivity and a satisfactory chromatographic baseline. As a result, under the optimized conditions, a baseline separation was achieved within 26 min, with symmetrical, sharp and well-resolved peaks for Hederacolchisid F.

The chromatogram is showing the complete baseline separation of Hederacolchisid F in the crude extract of Colchis ivy (**Fig. 3**). This method was validated according to the ICH guidelines on the validation of analytical methods [10]. No interference from other compounds but great resolution was observed for HCF and in the crude extract of Colchis ivy by the comparison of retention time. The peak purity was verified using HPLC (Agilent Chemstation software) and NMR methods.

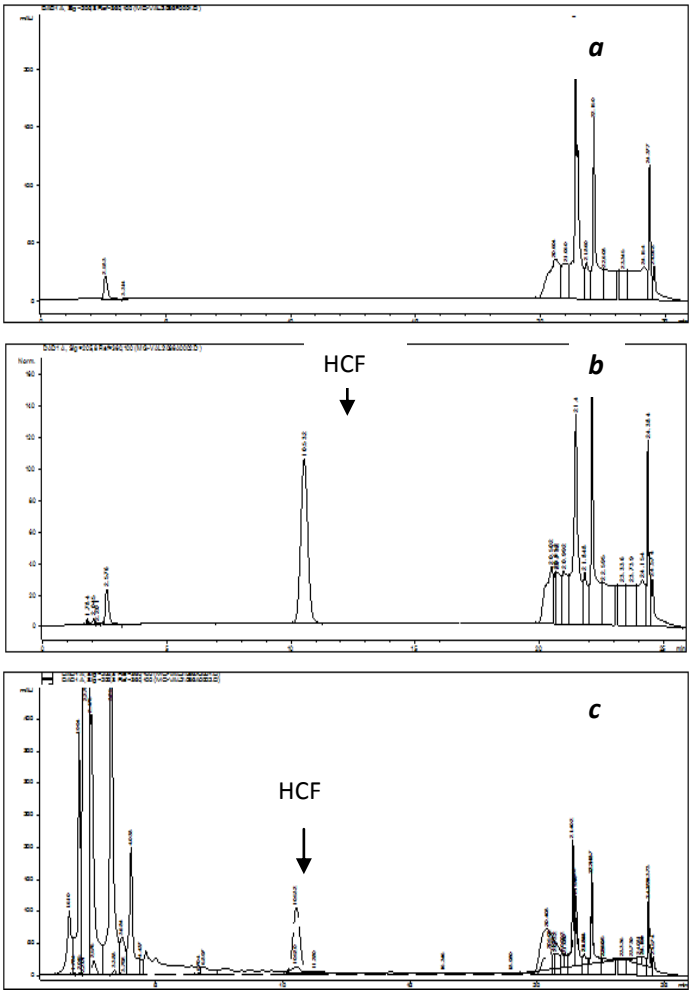

FIG.3: CHROMATOGRAPHIC PROFILES OF METHANOL (a); HEDERACOLCHISID F IN METHANOL (b); THE CRUDE EXTRACT OF COLCHIS IVY IN METHANOL (c).

We have managed to develop a quantitative method for the crude extract of Colchis ivy and validated a HPLC method for the assay of Hederacolchisid F. The calibration curves for the crude extract of Colchis ivy were linear within the concentration range (**Table1**). All correlation coefficients were greater than 0,999. The inter-day % RSD was less than 1.7 % and the intra-day % RSD was less than 1.04% for the crude extract of Colchis ivy which proves a good precision of the method (**Table 1**).

TABLE 1: CALIBRATION PARAMETERS, ACCURACY AND PRECISION OF HEDERACOLCHISID F IN THE CRUDE EXTRACT OF COLCHIS IVY.

Linearity	**Range (mg/ml)**	**0,002-1,0**	
	Calibration curves and r^2	$y = 1722x + 18,33$	
		$R^2 = 0,999$	
	Accuracy	99,8±0,9%	
Precision	Intermediate precision (Inter-day, 3 days) %RSD	First day	0.8962
		Second day	0.6436
		Third day	1.6061
	Intermediate precision (Intra-day) %RSD (n=18)	1,04	

The mean recoveries for Hederacolchisid F ranged from 97.8 to 102.62% **(Table 2).**

TABLE 2: ANALYTICAL RESULTS FOR RECOVERIES OF HEDERACOLCHISID F.

Relative amount added (%)	**Theoretical Concentration (mg/mL)**	**Found Concentration (mg/mL)**	**Recovery (%) (n=3)**
50	0,5	0,504	99,42
100	1.0	1,021	102,62
150	1,5	1,508	97,28

Recoveries determined for HCF ranged from 97.28 to 102.62% and RSDs were less than 1.03% indicating a good accuracy. The limit of detection was 0.0019 mg/mL. The limit of quantification was 0.0039 mg/mL. This validated HPLC method is successfully applied for the quantification of Hederacolchisid F in the crude extract (five batches) of Colchis ivy and is realized by external calibration (**Table 3**). The concentrations of HCF are homogeneous between the different samples. The content of HCF in the crude extract of Colchis ivy ranges from 1.43% to 1.77%.

TABLE 3: QUANTIFICATION OF BATCHES OF THE CRUDE EXTRACT OF COLCHIS IVY

Batch number	**Content of Hederacolchisid F %**
014066A	1.54
014066B	1.43
014066C	1.63
014066D	1.77
014066E	1.48

CONCLUSION: Finally, a new, simple, sensitive and reproducible HPLC method has been developed and validated for the simultaneous quantification of HCF in the crude extract of Colchis ivy. Precision, sensitivity and linearity are satisfactory in the range studied.

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