Chemistry of Natural Compounds, Vol. 41, No. 4, 2005

ECDYSTERONE AND TURKESTERONE IN *Ajuga turkestanica* DETERMINED BY HPLC

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UDC 543

Shoots of *Ajuga turkestanica* (Rgl.) Brig. (Labiatae, Mint) are a source of highly effective biologically active compounds such as phytoecdysteroids and natural glycosides [1, 2].

Ecdysterone, cyasterone, ajugalactone, ajugasterone, α-ecdysone, and turkesterone have been isolated previously from *A. turkestanica*. Of these, the main components are ecdysterone and turkesterone, which have very high anabolic activities [1].

The ecdysteroid content, in particular, that of turkesterone, in the raw material was determined by gravimetry [3-5] or UV spectrophotometry [6]. The turkesterone content varied from 0.025 to 0.17, which means that *A. turkestanica* is not an industrial raw material.

HPLC is known to determine with high accuracy the content of ecdysteroids in certain plant sources [7-9]. We investigated the contents of turkesterone and ecdysterone in the aerial part of *A. turkestanica* by HPLC on a Zorbax Eclipse XDB-C18 column, 3×150 mm, 3.5 μm (Agilent Technologies) using an Agilent LC 1100 chromatograph with a four-eluent pump, deaerator, and variable wavelength UV detector. The chromatograph was controlled and the results were processed using Agilent ChemStation software for the liquid chromatograph. UV detection of separate ecdysteroids was carried out at working wavelength 247 nm. The elution gradient is described below:

	Time, min					
	0.00	15.00	30.00	35.00	40.00	45.00
Water	100	95	15	0	0	100
Acetonitrile	0	5	85	100	100	0

The flow rate was 0.5 mL/min.

Ecdysterone and turkesterone were determined quantitatively using an external standard and a two-point calibration curve of the form

$$Y = mx + b,$$

where Y is the peak area and x is the amount of compound in %. Constants m and b were determined for turkesterone (m = 229310.00, b = 2.84×10^{-12}) and ecdysterone (m = 275180.00, b = 5.68×10^{-12}).

Standard solutions in ethanol (50%) at concentrations 0.1 and 0.01% were prepared for quantitative determination of ecdysteroids. Samples were introduced through a 20-μL loop on an Agilent manual injector.

The amount of dry substances in the extract was determined by refractometry. This enabled sample solutions to be prepared in the concentration range required for HPLC analysis. The analyzed sample was prepared at a concentration of 0.2% in ethanol (50%). The sample volume was 20 μL, as mentioned above. The same solvent gradient was used for elution. Ecdysteroids in the extract from *A. turkestanica* were identified and analyzed quantitatively using the Agilent ChemStation and the calibration table and curve (Fig. 1).

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0009-3130/05/4104-0475

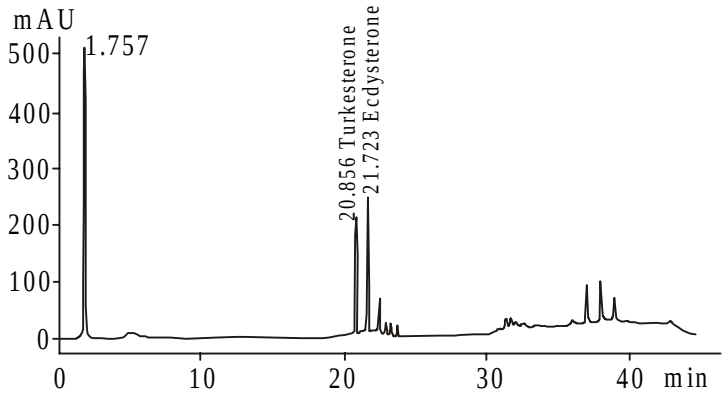

Fig. 1. HPLC chromatogram of *Ajuga turkestanica* extract (0.2% solution).

Considering the noticeable variation in climatic conditions at the plant sites, raw material collected in 1997, 2000, 2002, and 2004 during mass flowering, the first decade of May, was analyzed.

The investigations showed that the ecdysteroid content decreased gradually from 1997 to 2004. The contents of turkesterone (0.35%) and ecdysterone (0.38%) were highest in the material collected in 1997. By 2004, the content of turkesterone decreased to 0.22%; of ecdysterone, to 0.25%. In all probability, the main factor for this trend was a change of climate that included warming during the winter during these years and a relative increase in spring moisture.

Standard samples of ecdysterone and turkesterone were obtained by the literature method [4, 5].

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