



QUANTITATIVE ANALYSIS OF VALERENIC ACID IN HERBAL PRODUCTS ON BOSNIAN MARKET USING HPLC METHOD

Maja Pašić- Kulenović*, Bekir Behlulović and Larisa Alagić-Džambić

Development and Registration Department, Bosnalijek, Jukićeva 53, Sarajevo 71000, Bosnia and Herzegovina.

***Corresponding Author: Maja Pašić- Kulenović**

Development and Registration Department, Bosnalijek, Jukićeva 53, Sarajevo 71000, Bosnia and Herzegovina.

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ABSTRACT

Valerian (*Valeriana officinalis* L.) has been used in traditional medicine as a mild sedative and sleep-promoting agent. Sesquiterpenic acids expressed as valerenic acid consist of three fractions valerenic acid, cetoxyvalerenic acid and hydroxyvalerenic acid. These characteristic components of valerian are used as analytical markers to evaluate the quality of valerian preparations. Ten different valerian herbal products, in form of tincture, drops, capsules and film coated tablets available on the market have been tested on content of valerenic acids as analytical marker using high performance liquid chromatography (HPLC) method described in Valerian monographs of European Pharmacopoeia and United States Pharmacopoeia (USP).

KEYWORDS: *Valeriana officinalis*, valerenic acid, HPLC method.

INTRODUCTION

Valerian (*Valeriana officinalis* L, Caprifoliaceae) is a perennial flowering plant, with heads of sweetly scented pink or white flowers that bloom in the summer. Valerian flower extracts were used as a perfume in the 16th century.^[1] Crude extract of valerian root is sold as a dietary supplement in the form of capsules. Valerian root may have sedative and anxiolytic effects. The amino acid valine is named after this plant.

Known compounds detected in valerian that may contribute to its method of action are alkaloids (actinidine, chatinine, shyanthine, valerianine and valerine), isovaleramide (may be created in the extraction process), gamma-aminobutyric acid (GABA), isovaleric acid, iridoids, including valepotriates: isovaltrate and valtrate, sesquiterpenes (contained in the volatile oil): valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid, flavanones: hesperidin, 6-methylpigenin and linarin^[2,3,4]

The drug is often used as a milder alternative or a possible substitute for stronger synthetic sedatives, such as the benzodiazepines, in the treatment of states of nervous excitation and anxiety-induced sleep disturbances^[5], to relieve digestive and other spasms of smooth muscle. The valerian includes iridoids known as valepotriates, essential oil and non-volatile cyclopentane sesquiterpenes known as valerenic acid and derivatives.^[6]

European Pharmacopoeia (PhEur) has published monographs on valerian ground materials (Valerian root

and Valerian root, cut) and extracts (Valerian dry hydroalcoholic extract, Valerian dry aqueous extract and Valerian tincture). Content method is harmonised in all valerian monographs, but content of analytical marker substances are variable in different herbal drug preparations. Monograph for a finished medicinal product containing valerenic acids is not described. Pharmacopoeia (USP) has published monographs on Powdered valerian extract and Valerian tablets (Dietary Supplements Monograph, USP) with different HPLC method conditions.

The objective of the study was to investigate suitability of HPLC method described in PhEur monographs for quantification of sesquiterpenic acids (calculated as the sum of hydroxyvalerenic acid HVA, acetoxyvalerenic acid AOVA and valerenic acid VA, expressed as valerenic acid on dry basis.) in different dosage forms of finished medicinal products present on the Bosnian market.

MATERIAL AND METHODS

Ten different samples in duplicate were prepared and marked from Sample 1 to Sample 10. Samples from tinctures and drops were prepared using methanol as a solvent. Capsules and film coated tablets were pulverized into a powder, dissolved in methanol as a solvent, sonicated for 45 minutes, centrifuged for 10 minutes at 4000 rpm. All solution were filtered through a membrane nylon filter pore size 0.45 µm.

Chromatographic condition were:

- Stationary phase: L1; 250 x 4.6; 5 μ m, kept at 40°C
- UV detection at 220 nm
- Flow rate 1.5 ml/min (gradient elution system, Table 1)
- Injection volume 20 μ l
- Mobile phase:
 - mobile phase A: acetonitrile R1, 5 g/L solution of phosphoric acid R (20:80 V/V);
 - mobile phase B: 5 g/L solution of phosphoric acid R, acetonitrile R1 (20:80 V/V);

Table 1: Gradient system elution.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0 – 5	55	45
5 – 18	55 \rightarrow 20	45 \rightarrow 80
18 – 22	20	80
22.01	55	45
23 – 30	55	45

Standard solution was prepared in concentration 0.005 mg/ml. For linearity range standard solution were dilute from 20% to 200% of nominal target concentration. Acetonitrile HPLC grade purchased from Sigma-Aldrich and o-phosphoric acid 85% p.a. (Merck) were used for preparation of mobile phase. HPLC grade water was prepared by Milli-Q reverse osmosis Millipore (Bedford, MA, USA). Methanol (Sigma-Aldrich, HPLC grade) has been used as solvent. Reference standard (Valerian standardised dry extract) having 0.43% of sesquiterpenic acids (expressed as valerenic acid) was used as standard, and it was qualified using PhEur standard.

RESULT AND DISCUSSION

Linearity range results for HVA, AOVA and VC standards are given in Picture 1, 2 and 3. According to linearity results it can be concluded that the method is sensitive and linear for all three fractions (correlation coefficients for all fraction are 1.0000). Retention times are about 4 minutes for HVA, 10 minutes for AOVA and 18 minutes for VA. Assay content results (Table 2), show that four tested tinctures samples meet acceptance criteria for tinctures (min. 0.015% sesquiterpenic acids, expressed as valerenic acid). For sample 5, in form of drops, content of sesquiterpenic acids, expressed as valerenic acid, is less then acceptance criteria limit. Sample 6 content is dry hydroalcoholic extract and it assay content result is 0.284%, which meets acceptance criteria (min. 0.25% sesquiterpenic acids, expressed as valerenic acid). Samples 7, 8 and 9 are dry extracts, and they also meets acceptance criteria (min. 0.6% sesquiterpenic acids, expressed as valerenic acid for dry extract), correspond to 0.733%, 0.804% and 0.580%. Sample 10 is dry aqueous extract and it also meets acceptance criteria (min. 0.02% sesquiterpenic acids, expressed as valerenic acid for dry extract), correspond to 0.115%.

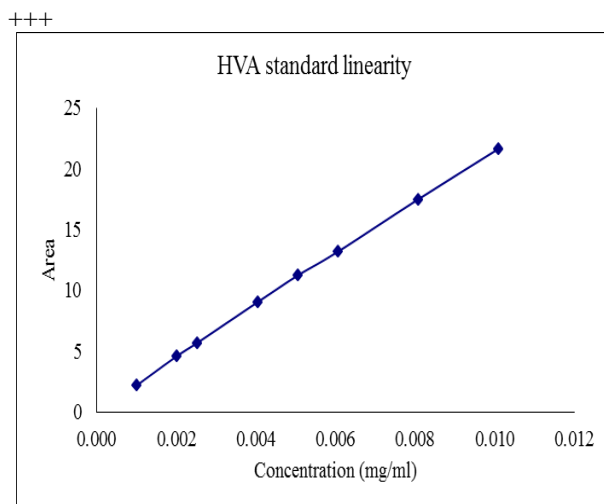


Figure 1: Linearity range for HVA standard solution.

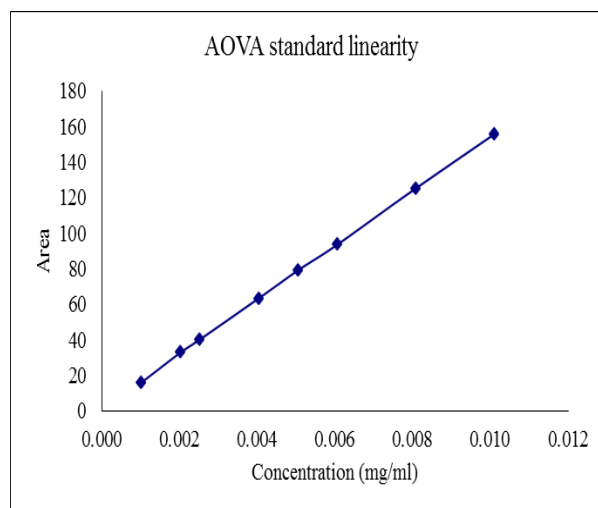


Figure 2: Linearity range for AOVA standard solution

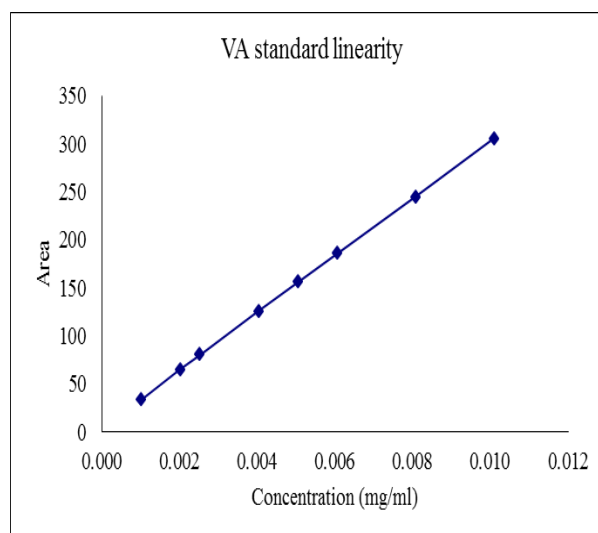
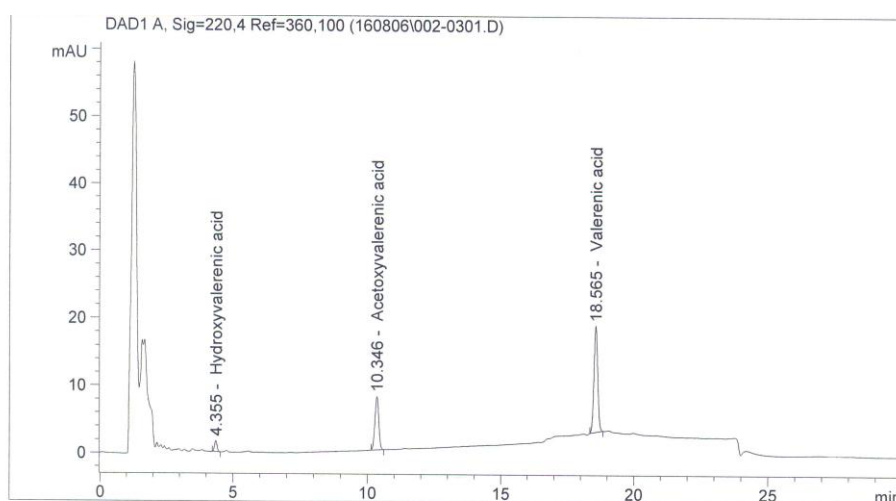


Figure 3: Linearity range for VA standard solution.

Table 2: Results of assay content of samples present on Bosnian market.

Sample No.	Area HVA	Area AOVA	Area VC	Area Σ	(%) valerenic acid	Acceptance criteria limits*
Sample 1- tincture	5.71908	39.1506	49.75367	94.62335	0.151	Min.0.015%
Sample 1- tincture	5.68456	39.6019	49.39341	94.67987	0.149	
Average value					0.150	
Sample 2- tincture	13.93978	32.76649	54.93805	101.64432	0.168	Min.0.015%
Sample 2- tincture	14.00883	33.33578	55.47996	102.82457	0.170	
Average value					0.169	
Sample 3- tincture	4.66008	41.23287	46.43602	92.32897	0.140	Min.0.015%
Sample 3- tincture	4.85766	41.63137	47.0444	93.53343	0.142	
Average value					0.141	
Sample 4- tincture	18.96725	15.22227	24.20947	58.39899	0.065	Min.0.015%
Sample 4- tincture	19.0698	15.20872	23.18942	57.46794	0.062	
Average value					0.063	
Sample 5- drops	0	0	5.12074	5.12074	0.001	Min.0.015%
Sample 5- drops	0	0	8.80008	8.80008	0.013	
Average value					0.007	
Sample 6- tablets	10.47469	50.75494	93.75996	154.98959	0.284	Min.0.25%
Sample 6- tablets	10.3405	51.28234	93.59928	155.22212	0.283	
Average value					0.284	
Sample 7- tablets	23.10932	167.2395	330.23291	520.58173	0.727	Min.0.6%
Sample 7- tablets	23.46232	170.0435	335.44968	528.9555	0.739	
Average value					0.733	
Sample 8- capsules	16.14602	150.2206	245.06718	411.4338	0.805	Min.0.6%
Sample 8- capsules	16.31434	149.50137	244.63982	410.45553	0.804	
Average value					0.804	
Sample 9- capsules	13.82063	79.49821	107.76716	201.086	0.575	Min.0.6%
Sample 9- capsules	14.6216	81.32654	109.5386	205.48674	0.585	
Average value					0.580	
Sample 10- tablets	13.84699	56.04724	56.79973	126.69396	0.116	Min.0.02%
Sample 10- tablets	13.97553	56.53794	55.55646	126.06993	0.113	
Average value					0.115	

*acceptance criteria according to European Pharmacopoeia and United States Pharmacopoeia

**Figure 4: Chromatogram for sesquiterpenic acids standard solution.****CONCLUSION**

Application of HPLC method 1 described in PhEur for valerenic powder and extract was successfully applied for quantification of sesquiterpenic acids in different dosage forms of marketed herbal products.

Tested products on Bosnian market in form of tincture, drops and capsules have contained acceptable range of valerenic acid. Sample of film coated tablets containing valerenic acid contained lower content than the one declared on the package insert.

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