*ejpmr, 2018,5(5), 100-103*

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

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

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**Article Received on 08/03/2018** **Article Revised on 29/03/2018** **Article Accepted on 18/04/2018**

## 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-methylapigenin 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 naylon filter pore size 0.45 µm.

Chromatografic condition were:

- Stationary phase: L1; 250 x 4.6; 5 µm, kept at 40°C
- UV detection at 220 nm
- Flow rate 1.5 ml/min (gradient eluation system, Table 1)
- Injection volume 20 µl
- 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 eluation.**

| Time (min) | Mobile phase A (% v/v) | Mobile phase B (% v/v) |
|---|---|---|
| 0 – 5 | 55 | 45 |
| 5 – 18 | 55→20 | 45→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 hydroalcocholic axtract 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%.

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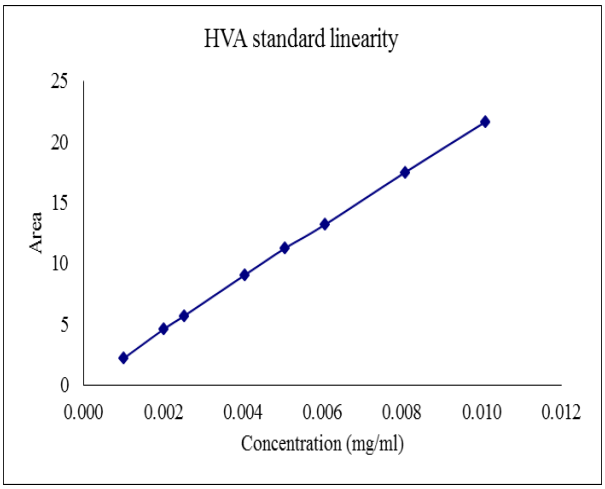


**Figure 1: Linearity range for HVA standard solution.**

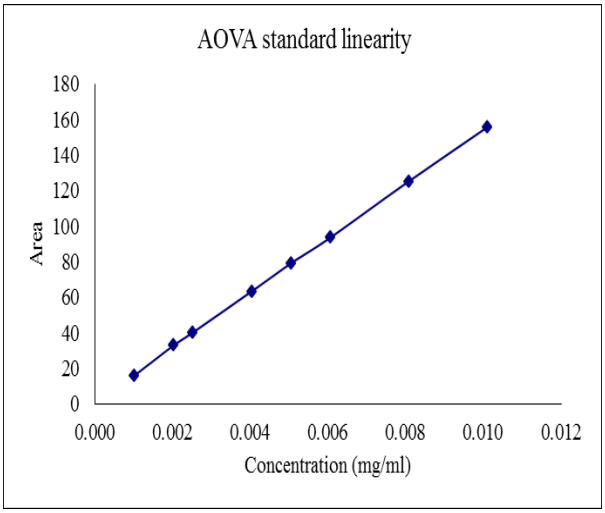


**Figure 2: Linearity range for AOVA standard solution**

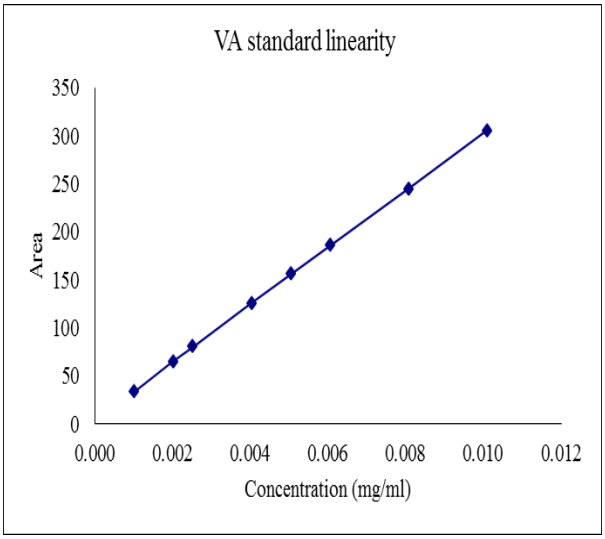


**Figure 3: Linearity range for VA standard solution.**

**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 | |
| Sample 1- tincture | 5.68456 | 39.6019 | 49.39341 | 94.67987 | 0.149 | Min.0.015% |
| Average value | | | | | **0.150** | |
| Sample 2- tincture | 13.93978 | 32.76649 | 54.93805 | 101.64432 | 0.168 | |
| Sample 2- tincture | 14.00883 | 33.33578 | 55.47996 | 102.82457 | 0.170 | Min.0.015% |
| Average value | | | | | **0.169** | |
| Sample 3- tincture | 4.66008 | 41.23287 | 46.43602 | 92.32897 | 0.140 | |
| Sample 3- tincture | 4.85766 | 41.63137 | 47.0444 | 93.53343 | 0.142 | Min.0.015% |
| Average value | | | | | **0.141** | |
| Sample 4- tincture | 18.96725 | 15.22227 | 24.20947 | 58.39899 | 0.065 | |
| Sample 4- tincture | 19.0698 | 15.20872 | 23.18942 | 57.46794 | 0.062 | Min.0.015% |
| Average value | | | | | **0.063** | |
| Sample 5- drops | 0 | 0 | 5.12074 | 5.12074 | 0.001 | |
| Sample 5- drops | 0 | 0 | 8.80008 | 8.80008 | 0.013 | Min.0.015% |
| Average value | | | | | **0.007** | |
| Sample 6- tablets | 10.47469 | 50.75494 | 93.75996 | 154.98959 | 0.284 | |
| Sample 6- tablets | 10.3405 | 51.28234 | 93.59928 | 155.22212 | 0.283 | Min.0.25% |
| Average value | | | | | **0.284** | |
| Sample 7- tablets | 23.10932 | 167.2395 | 330.23291 | 520.58173 | 0.727 | |
| Sample 7- tablets | 23.46232 | 170.0435 | 335.44968 | 528.9555 | 0.739 | Min.0.6% |
| Average value | | | | | **0.733** | |
| Sample 8- capsules | 16.14602 | 150.2206 | 245.06718 | 411.4338 | 0.805 | |
| Sample 8- capsules | 16.31434 | 149.50137 | 244.63982 | 410.45553 | 0.804 | Min.0.6% |
| Average value | | | | | **0.804** | |
| Sample 9- capsules | 13.82063 | 79.49821 | 107.76716 | 201.086 | 0.575 | |
| Sample 9- capsules | 14.6216 | 81.32654 | 109.5386 | 205.48674 | 0.585 | Min.0.6% |
| Average value | | | | | **0.580** | |
| Sample 10- tablets | 13.84699 | 56.04724 | 56.79973 | 126.69396 | 0.116 | |
| Sample 10- tablets | 13.97553 | 56.53794 | 55.55646 | 126.06993 | 0.113 | Min.0.02% |
| Average value | | | | | **0.115** | |

**acceptance criteria according to European Pharmacopoeia and United States Pharmacopoeia*

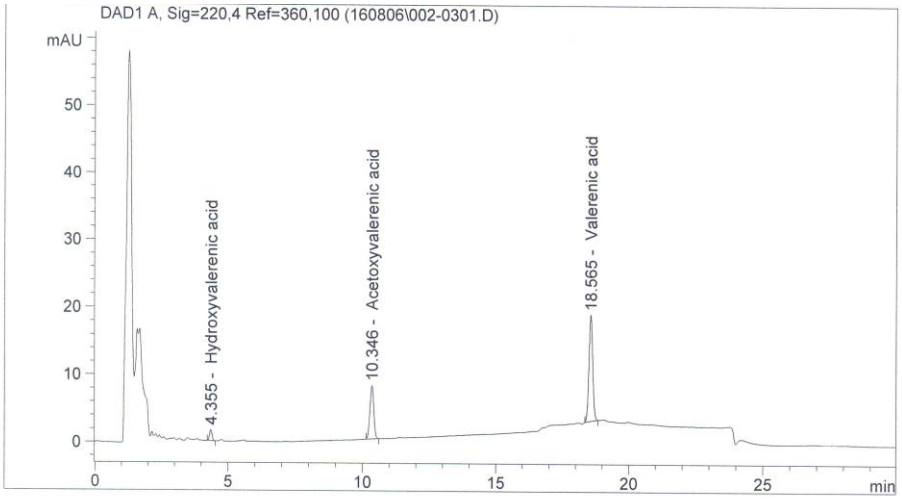


**Figure 4: Chromatogram for sesquiterpenic acids standard solution.**

## CONCLUSION

Application of HPLC method 1 described in PhEur for velerenic 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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