

## Quantification of Valerenic Acid in Sleep and Relax® Herbal Product

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*Valeriana officinalis*, well known as valerian, is one of the important herbal medicines, belongs to Valerianaceae family. The valerian genus covers around 250 species throughout Europe and Asia. Six of the *Valeriana* species grow wildly in Iran. The anxiolytic and sedative effect of valerian is the valuable medicinal properties of this plant and would be due to the presence of valerenic acid. For this reason, quantitation of valerenic acid is very important in herbal remedies and preparations contained valerian extract. In the present study, quantitation of valerenic acid has been reported in a phytopharmaceutical product, Sleep&Relax®, which is widely exhibited in drugstores and herbal pharmacies across Canada and Iran. The result of this study shows that the average amount of valerenic acid evaluated was 40 mg in each capsule which is in agreement with the claimed amount on the brochure of product. The percentage of valerenic acid in this product has been measured as 113.43% which is in agreement with the USP standards (90% -120%). The measured amounts of valerenic acid were decreased as 51% for one month and 53% for 6 month storages compared to the samples stored at room temperature and normal condition.

**Key words:** Valerenic acid, Valerian, HPLC.

Nowadays, herbal medicines are widely used throughout developing and developed countries as over-the counter-drugs (OTC), home remedies, and also as crude materials for preparing herbal pharmaceutical companies. Valerian, *Valeriana officinalis*, is one of these valuable herbal medicines belonging to Valerianaceae family<sup>1-3</sup>.

*Valeriana* genus is native to Europe and Asia and has over 250 species throughout the world. Six species of them are wildly growing in Iran as followed: *V. alliarifolia*, *V. clarkei*, *V. ficariifolia*, *V. leucophaea*, *V. officinalis* and *V. sisymbriifolia*<sup>3</sup>.

Traditionally, dried roots of this plant (root powder or extract) have been used for preparing medicinal teas, and also modern dosage forms such as capsules and tablets which are usually administered for psychotic diseases *e.g.* insomnia, anxiety, restlessness and dyssomnia<sup>4-6</sup>. The root of *V. officinalis* contains two main groups of compounds. The first is sesquiterpenes found in the volatile oil (valerenic acid and its derivatives, valeranone, valeranal and kessyl esters) and the

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second isvalpotriates (valtrate, didrovaltrate, acevaltrate, ovaleroxyhydroxyvaltrate). Furthermore, flavonoids, triterpenes, lignans and alkaloids have been reported from this species<sup>7</sup>. Valerenic acid, with molecular formula  $C_{15}H_{22}O_2$ , is the main active ingredient of valerian extract (Fig. 1). The anxiolytic and sedative effects of valerian are demonstrated *via* gamma-amino butyric acid (GABA) ergic mechanisms. Previous studies showed that valerenic acid can bind to GABA receptors and modulate the GABA<sub>A</sub> receptor function<sup>8</sup>.

So far, HPLC, TLC and HPTLC are commonly used for detection, quantitative and qualitative analysis of valerenic acid in *Valeriana* species<sup>7,9-10</sup>. The HPTLC method is either suitable for rapid screening of a large number of plant samples, or useful for crop improvement under the plant-breeding program (valerian). The HPLC method developed here for quantitation of valerenic acid is rapid, cost-effective and easily adaptable for screening and quantitative analysis of valerian extracts compared to other analytical techniques. In this paper, we aimed to detect and quantify (using HPLC) valerenic acid in a phytopharmaceutical product, named Sleep & Relax®, produced by HLC-Healinglinecoro of Canada. This product is widely exhibited and easily found in drugstores and herbal pharmacies across Canada and also Iran.

## MATERIALS AND METHOD

### Sleep & Relax capsule

This capsule contains extracts of hops (*Humulus lupulus*), lemon balm (*Melissa officinalis*), Passion flower (*Passiflora incaranta*), skullcap (*Scutellaria lateriflora*) and valerian (*V. officinalis*). The root extract of valerian in this capsule should contain valerenic acid about 0.8% w/w on the basis of the product's brochure.

## EXPERIMENTAL

The HPLC used in this analysis is Knauer Corp®. Its column packed with Eurospher 100-5 C18 in dimension 250×4mm. The detector is photodiode array (PDA) 2600 with Deuterium lamp E4350 model and pump is Smart line 1000, including 10 mL pump head stainless steel EA4300 model.

The mobile phases were considered as deionized water and methanol. The flow rate was 1 mL/min. Valerenic acid was used as a standard compound, purchased from Fluka Corp® with purity more than 98.0% (HPLC).

### Sample preparation

Contents of twenty capsules were weighed exactly, dissolved in 50 mL methanol and transferred into a volumetric flask, then stirred and sonicated for 30 minutes at 30°C. The sample was filtered by a filter paper and injected (20 µl) to HPLC. Detected was performed with UV detector at 225 nm using below gradient program Table 1.

### Standard preparation

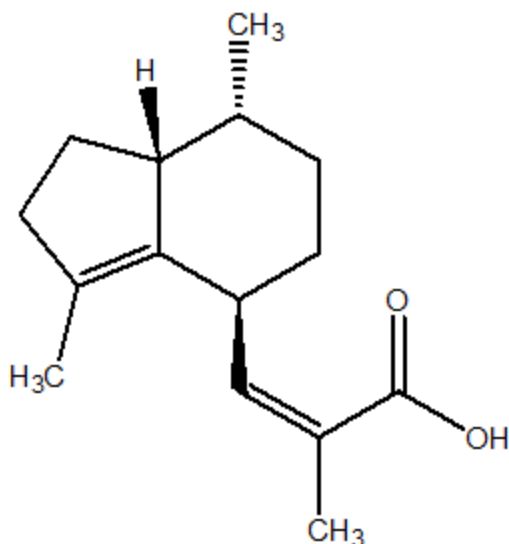
In order to plot the calibration curve of valerenic acid, four concentrations of external standards were used. Two milligrams of the standard powder was dissolved in 5 mL of methanol. Serial dilutions were prepared as 0.4, 0.2, 0.1 and 0.05 mg/mL. Each of the mentioned concentrations was injected to HPLC two times at 25 °C and 225 nm. HPLC elution condition was similar to the program used in the section three. The calibration graph showed a linear correlation between sample concentration and peak area<sup>11</sup>. The regression equation was  $18961x - 383.48$ . The correlation coefficient (r) and limit of detection (LOD) were calculated as 0.993 and 0.015 mg/mL, respectively Fig. 2.

### Heat-Stability studies

For heat-stability studies two states of the samples were chosen and stored at 40°C and humidity of 75%. The first state was studied after 1 month but the second state was analyzed after 6 month.

**Table 1.** HPLC Elution Condition at 25 °C of valerenic acid from Sleep& Relax® herbal product.

CH <sub>3</sub> OH%	H <sub>2</sub> O%	Flow (ml/min)	Time (min)
20	80	1	0
20	80	1	10
100	0	1	11
100	0	1	30



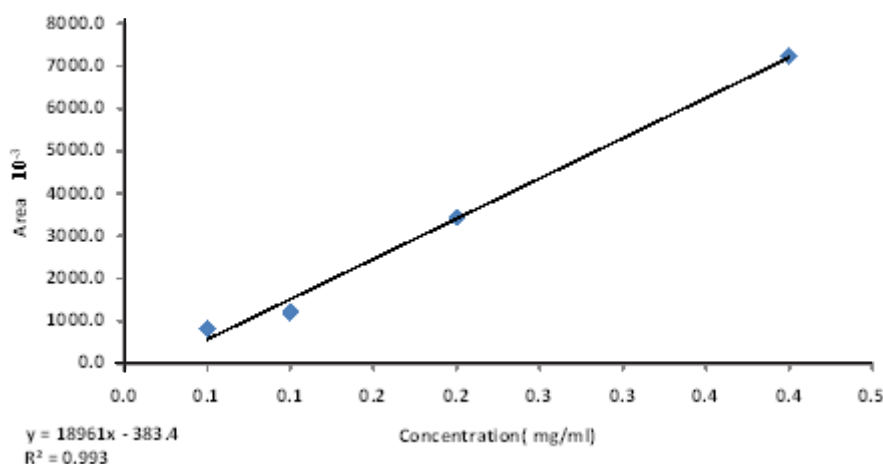
**Fig. 1.** Chemical structure of the compound, valerenic acid

## RESULTS AND DISCUSSION

In this study, the total contents of 20 capsules were analyzed by using HPLC in order to quantify the percentage of valerenic acid in the phytopharmaceutical product Sleep & Relax®. Four dilutions of the standard, valerenic acid were used for plotting a calibration curve and obtaining

the exact concentration of valerenic acid inside the product samples. The result showed that the average amount of valerenic acid has been evaluated as 40 mg in each capsule which is in agreement with the claimed amount on the brochure of product. On the basis of the USP standards, acceptable percentage for valerenic acid should be between 90% -120%. This was calculated as 113.43% for the normal samples of the product which is inside the acceptable ranges<sup>12</sup>. The results of heat-stability studies showed that the measured amounts of valerenic acid were decreased as 51% for one month and 53% for 6 month storages compared to the samples stored at room temperature and normal condition. It is reported that the valerian extract is sensitive to the temperature of 50°C. This is mainly attributed to reduction of valepotriates in the sample but the reduction of valerenic acid has been reported less often than valepotriates<sup>11</sup>. The previous studies revealed that valerian root powder should be stored at a low to moderate temperature in order to conserve the valerenic acid safely, but determination of the optimum temperature requires further studies of storage between the ranges of 14-30°C<sup>13</sup>.

In conclusion, valerian is now used for sedative and psychotic diseases. Literature review reveals that valerian can improve natural sleeping if consumes for several weeks without addiction or considerable adverse effects. The sedative and



**Fig. 2.** Calibration curve for standard of valerenic acid.

anticonvulsant properties of the valerian remedies are reported to be related to valerenic acid<sup>14</sup>. Detection and quantitation of valerenic acid in Sleep&Relax®, as a phytopharmaceutical product, resulted in the acceptable percentage of valerenic acid inside a sample stored at normal condition. It seems that the company should highlight a caution about the storage temperature of the product at home or drugstores.

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