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Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0068 - Valerian for Valerenic Acids by HPLC

Botanical Name: *Valeriana officinalis L.*

Common Names: Garden heliotrope, garden valerian

Parts of Plant Used: Subterranean parts including the rhizomes and roots.

Uses: Treatment of anxiety, sleep disorders, and insomnia.

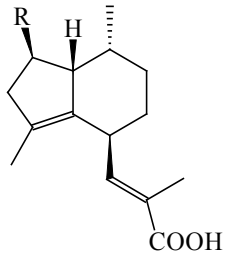
Modes of Action:

Various clinical trials with valerian have shown positive effects on anxiety, sleep disorders, and mood. Multiple compounds in valerian are believed to be responsible for its activities. These components include iridoids (valepotriates) and their degradation products (baldrinals), sesquiterpenes (valerenic acid, hydroxyvalerenic acid, acetoxyvalerenic acid), essential oil, and even flavonoids (6-methylapigenin, hesperidin). Animal studies have shown that valerenic acid may inhibit enzymes that break down γ-aminobutyric acid (GABA), increasing GABA levels and producing a CNS-depressing effect. In addition, 6-methylapigenin is a benzodiazepine binding site (BDZ-bs) ligand of the GABAA receptor.[1,2] These two valerian compounds may work synergistically to produce a sedative effect.

Chemical Markers:

Numerous compounds have been identified in valerian. The main ones are iridoids (the iridoid esters valtrate, acevaltrate, isovaltrate, and dihydrovaltrate are very unstable and can be degraded easily by moisture and heat to baldrinal and isopropylbaldrinal), sesquiterpenes (valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid), flavonoids (hesperidin, 6-methylapigenin, glycoside derivatives of quercetin, diosmetin, luteolin, apigenin, kaempferol, and acacetin), alkaloids, amino acids, sterols, and tannins.[2–4]

When the essential oil of valerian was studied by GC and GC–MS, the main components were found to be bornyl acetate, spathulenol, valeranone, valerenal, kessyl acetate, and (–)-valerena-4,7(11)-diene.5 As valerenic acids are bioactive compounds in valerian and stable, they are used as chemical markers for the quality control of valerian root powders and extracts.



Valerenic acid: R = H
Hydroxyvalerenic acid: R = OH
Acetoxyvalerenic acid: R = OAc
Major valerenic acids found in valerian.

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Methods of Analysis

HPLC has been used for the analysis of three types of compounds (valerenic acids, valepotriates, baldrinals) with the valerenic acids used as the quality control standards. Valerenic acids can be extracted easily with 50% ethanol, methanol, and 60% acetonitrile through water-bath heating or sonication.

Method 1:

The HPLC method of Bokstaller and Schmidt[6] was used for analysis.

Sample Preparation:

Prepare samples in 50% ethanol.

Chromatography:

Column: Merck LiChrospher RP100 C18, 5 µm, 250 × 4.0 mm with a guard column (Merck LiChrospher RP100 C18, 5 µm, 4.0 × 4.0 mm).
Mobile phase: Solvent A = acetonitrile–water (110:690 vol/vol) solvent B = acetonitrile–water (120:180 wt/wt). Both solutions were adjusted to pH 2.8 with 85% phosphoric acid.
Gradient:

Time (minutes)	%A	%B
0	55	45
5	55	45
10	25	75
15	25	75
20	0	100
22	0	100
22.5	55	45

Flow rate: 1.5 mL/minute
Injection volume: 10 µL
Detection wavelength: 225 nm

Validation Data:

Not available

Method 2:

The ChromaDex HPLC method was used.

Sample Preparation:

Weigh about 1 g of 0.8% valerenic acid extract into a 100-mL volumetric flask, add about 50 mL of 60% acetonitrile, and sonicate for 15 minutes. Then cool to room temperature and dilute to volume with 60% acetonitrile.

Chromatography:

Column: Phenomenex Luna C18 (2), 5 µm, 4.6 × 150 mm.
Mobile phase: Solvent A = acetonitrile–water–85% phosphoric acid (40:60:0.2), solvent B = acetonitrile–water–85% phosphoric acid (80:20:0.2).
Gradient:

Time (minutes)	%A	%B
0	100	0
20	0	100

Flow rate: 1.5 mL/minute
Injection volume: 10 µL
Detection wavelength: 218 nm
Column temperature: Ambient

Validation Data:
Not available

Method 3:
The unpublished method of Mingfu Wang was used for the HPLC determination of valerenic acids.

Sample Preparation:
Extract about 200 mg of 0.8% valerenic acid extract with 35 mL of 60% methanol using sonication for 25 minutes. Then cool to room temperature, and dilute to volume with methanol.

Chromatography:
Column: Phenomenex Prodigy ODS (3), 5 µm, 150 × 3.2 mm.
Mobile phase: Solvent A = water (adjusted to pH 2.5 with phosphoric acid), solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B
0	60	40
8	56	44
22	24	76
27	24	76
27.1	60	40
40	60	40

Flow rate: 0.9 mL/minute
Injection volume: 10 µL
Detection wavelength: 214 nm
Column temperature: Ambient

Method 4:
The method of Bos et al.[7] can be used to analyze three types of compounds simultaneously: valerenic acids, valepotriates, and baldrinals.

Sample Preparation:
Weigh 1.5 g of ground material, add 20 mL of methanol, and reflux in a water bath for 30 minutes. Filter into a 50-mL volumetric flask, add an additional 20 mL of methanol, and reflux for 15 minutes more. Combine the extraction solutions, cool to room temperature, and dilute to volume with methanol.

For capsules, cut capsules, extract the material with three portions of 30 mL of methanol for 5 minutes using sonication. Combine the extraction solutions into a 100-mL volumetric flask, and dilute to volume.

For tablets, grind five tablets together and extract twice with 10 mL of methanol using sonication for 5 minutes. Transfer the extraction solution to a 25-mL volumetric flask and dilute to volume.

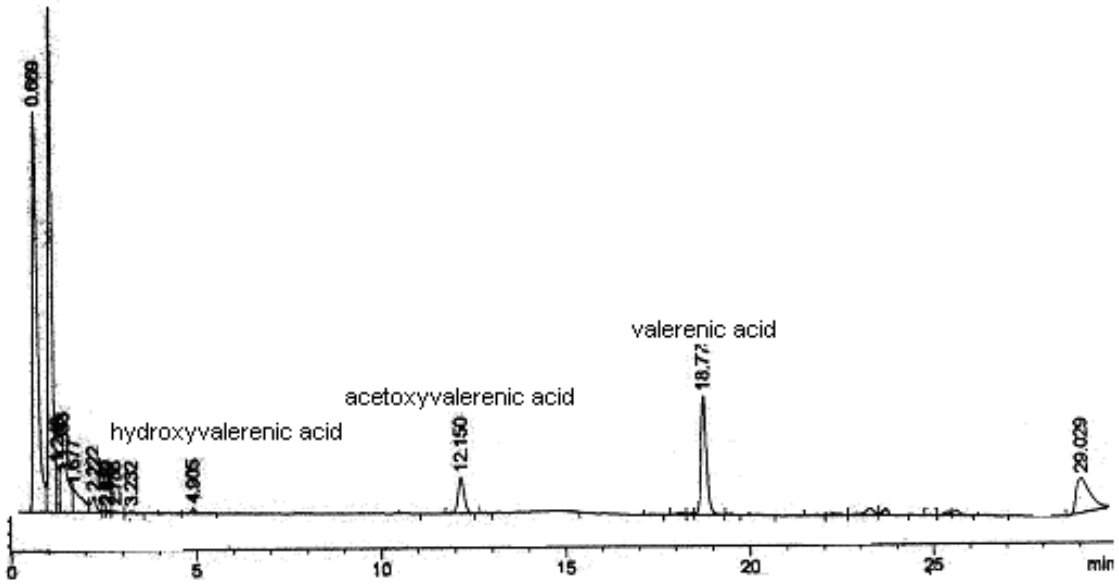
Chromatography:
Column: Superspher RP100 C18 (5 µm, LiChroCART 250 × 4.0 mm) with guard column (Merck LiChrospher RP100 C18, 5 µm, LiChroCART 4.0 × 4.0 mm).
Mobile phase: Solvent A = water–acetonitrile (800:156.4 wt/wt), solvent B = water–acetonitrile (200:625.6 wt/wt) (both eluents contain 1 mM phosphoric acid).

Gradient:

Time (minutes)	%A	%B
0	55	45
5	55	45
24	0	100
26	0	100
28	55	45
33	55	45

Flow rate: 1.5 mL/minute
Column temperature: 25°C
Injection volume: 20 µL
Detection wavelength: 256 nm for valtrate, isovaltrate, and acevaltrate; 203 nm for didrovaltrate and isovaleroxyhydroxydidrovaltrate; and 230 and 246 nm for baldrinal and homobaldrinal.

Representative HPLC Chromatogram of Valerian Run by Method 3



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