Supporting Information

Anxiolytic Properties of *Valeriana officinalis* in the Zebrafish: A Possible Role for Metabotropic Glutamate Receptors
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Affiliation
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Correspondence
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Department of Pharmacology and Toxicology
PO Box 365067 San Juan
Puerto Rico 00936-5067
lisamdelvalle@yahoo.com; lisa.delvalle@upr.edu
PRODUCT NAME: Valerian Root-Certified Organically Grown
BOTANICAL NAME: Valeriana officinalis
PRODUCT LOT#: 1020P-OUF
ORIGIN: USA (Harvested: 2008)
PLANT PART AND FORM: Roots, fresh & whole
ORGANIC CERTIFICATION: Oregon Tilth
EU CERTIFICATION: Oregon Tilth
KOSHER CERTIFICATION: Earth Kosher
GMO FREE

DESCRIPTION:
A. Organoleptic:
   1) Color, Odor and Flavor: Whitish color, characteristic, strong sweet, musty odor and flavor
   2) Extraneous Matters: None

B. Chemical:
   1) Moisture Content: N/A
   2) Steam Volatile Oil: N/A
   3) Sulfite: N/A
   4) Pesticide & Chemical Residues: N/A
   5) Others:

C. Microbiological:
   1) Total Aerobic Plate Count: N/A
   2) Yeast & Mold: N/A
   3) Pathogens: N/A

D. General Shipping Guidelines:
   1) In new cardboard boxes with appropriate labels
To the best of our knowledge, there has been no use of chemicals, sewage sludge, genetically modified materials or radiation of any type in the cultivation, harvest, drying or shipping of this product.

Approved by: Lisa Ward
Date issued: 08/25/10
Crop List Addendum for Pacific Botanicals

Certification Number OR-OTCO-CO-84-00084

Organic Crops:
- Alfalfa (Medicago sativa)
- Angelica (Angelica archangelica)
- Artemisia (Artemisia annua)
- Artichoke (Cynara scolymus)
- Ashwagandha (Withania somnifera)
- Astragalus (Astragalus mongholicus membr.)
- Bacopa (Bacopa monnieri)
- Basili-Turhi (Ocimum sanctum)
- Black Walnut (Juglans nigra)
- Blessed Thistle (Cnicus benedictus)
- Blue Cohosh (Caulophyllum thalictroides)
- Borage (Borago officinalis)
- Burdock (Arctium lappa)
- Calendula (Calendula officinalis)
- California Poppy (Eschscholzia californica)
- Catnip (Nepeta cataria)
- Colandine (Chelidonium majus)
- Chamomile (Matricaria recutita)
- Cherry (Prunus spp.)
- Corn (Zea mays)
- Currant-Black (Ribes nigrum)
- Dandelion (Taraxacum officinale)
- Echinacea (Echinacea purpurea)
- Eclipta (Eclipta alba)
- Elderberry (Sambucus spp.)
- Elecampane (Inula helenium)
- Eleuthero (Eleutherococcus senticosus)
- Fennel (Foeniculum vulgare)
- Feverfew (Tanacetum parthenium)
- Fig (Ficus carica)
- Garlic (Allium sativum)
- Gentian (Gentiana lutea)
- Ginkgo (Ginkgo biloba)
- Gladiolus (Gladiolus spp.)
- Goji Berries (Centella asiatica)
- Grape (Vitis spp.)
- Horse Chestnut (Aesculus hippocastanum)
- Hyssop (Hyssopus officinalis)
- Lavender (Lavandula spp.)
- Lemon Balm (Melissa officinalis)
- Licorice (Glycyrrhiza glabra)
- Linden (Tilia cordata)
- Lobelia (Lobelia inflata)
- Marshmallow (Althaea officinalis)
- Milk Thistle (Silybum marianum)
- Motherwort (Leonurus cardiaca)
- Mullein (Verbascum olympicum and V. thapsus)
- Nettles (Urtica dioica)
- Oat (Avena sativa)
- Oregon Grape (Mahonia aquifolium)
- Plantain (Plantago spp.)
- Pokeweed (Phytolacca americana)
- Raspberry (Rubus idaeus)
- Red Clover (Trifolium pratense)
- Rosemary (Rosmarinus officinalis)
- Rye (Secale cereale)
- Sage-White (Salvia apiana)
- Sage-Gardan (Salvia officinalis)
- Sheep Sorrel (Rumex acetosella)
- Skullcap (Scutellaria baicalensis)
- Skullcap (Scutellaria lateriflora)
- Spilanthes (Spilanthes acmella)
- Valerian (Valeriana officinalis)
- Vital (Vitis augs-castus)
- Wood Betony (Stachys officinalis)
- Wormwood (Artemisia absinthium)
- Yarrow (Achillea milfolium)
- Yellow Wild Indigo (Baptisia tinctoria)

Certified by OTCO since: 7/1/1984
NOP effective date: 4/29/2002
Analytical Test Report

Customer: University of Puerto Rico MSC
Report Number: CDXA-ATR-2959-00

Address (City, State): San Juan, P.R.
Purchase Order: 440009651
Date of Report: 7-Feb-11

Assay: Valerian for Valerenic Acids by HPLC
Part Number: CDA-00021001-ATR

Prepared By: Richard Vigil
Analytical Chemist
7-Feb-11

Reviewed By: Maria Bialecki
Quality Assurance
7-Feb-11

Approved By: Kimberly Eastman
Manager, Analytical Services
7-Feb-11

Signed original on file at CDXA

This product analysis is subject to our “Standard Terms and Conditions for the Purchase and Sale of ChromaDex Products and or Services,” a copy of which has been provided to our client and is incorporated herein by this reference. As more specifically set forth therein, this product analysis is for the benefit of our client only, may not be relied upon by any other party without our prior written consent, relates solely to the sample(s) provided to us by our client and therefore cannot be applied to any other material or sample.
SUMMARY

- **SAMPLE(S)**
  - **Lot #**
    - Valeriana Officinalis 2004: Not Provided (111H-OUP)
    - Valeriana Officinalis 2008: Not Provided (1020P-OUP)
  - **CDXA #**
    - CDXA-11-0596
    - CDXA-11-0597

- **RESULTS**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Spec.</th>
<th>Result</th>
<th>Reporting Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyvaleric Acid</td>
<td>%</td>
<td></td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Acetoxyvaleric Acid</td>
<td>%</td>
<td></td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td>Valeric Acid</td>
<td>%</td>
<td></td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>N/A</td>
<td>0.293</td>
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<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Spec.</th>
<th>Result*</th>
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</thead>
<tbody>
<tr>
<td>Hydroxyvaleric Acid</td>
<td>mg/g</td>
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<td>0.21</td>
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<tr>
<td>Acetoxyvaleric Acid</td>
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<td></td>
<td>1.19</td>
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<tr>
<td>Valeric Acid</td>
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<tr>
<td>Total</td>
<td>mg/g</td>
<td>N/A</td>
<td>2.93</td>
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<thead>
<tr>
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<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Hydroxyvaleric Acid</td>
<td>%</td>
<td></td>
<td>BRL</td>
<td>0.007</td>
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<tr>
<td>Acetoxyvaleric Acid</td>
<td>%</td>
<td></td>
<td>ND</td>
<td>0.008</td>
</tr>
<tr>
<td>Valeric Acid</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>N/A</td>
<td>0.016</td>
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</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Spec.</th>
<th>Result*</th>
<th>Reporting Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyvaleric Acid</td>
<td>mg/g</td>
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<td>BRL</td>
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<tr>
<td>Acetoxyvaleric Acid</td>
<td>mg/g</td>
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<td>0.160</td>
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<tr>
<td>Valeric Acid</td>
<td>mg/g</td>
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<tr>
<td>Total</td>
<td>mg/g</td>
<td>N/A</td>
<td>0.160</td>
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</table>

*Customer requested reporting in mg/mL, however, sample was a solid, hence the reported results are per g of solid material

ND – Not detected above reporting Limit
BRL – Below reporting limit (compound detected below RL)
ANALYTICAL METHOD

- **STANDARD(S)** All standards supplied by ChromaDex; unless otherwise specified.

<table>
<thead>
<tr>
<th>Part #</th>
<th>Standard</th>
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<tbody>
<tr>
<td>ASB-00001060</td>
<td>Acetoxyvaleric Acid</td>
</tr>
<tr>
<td>ASB-00008730</td>
<td>Hydroxyvaleric Acid</td>
</tr>
<tr>
<td>ASB-00022150</td>
<td>Valeric Acid</td>
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</tbody>
</table>

- **LABORATORY SUPPLIES**
  - Analytical Balance
  - Stir Plate
  - Assorted and Volumetric glassware
  - Syringes and Syringe Filters
  - HPLC/GC glass vials and caps
  - Vacuum System
  - Whatman Filters
  - Blender
  - Centrifuge

- **SOLVENTS AND REAGENTS**
  - Acetonitrile (ACN)
  - Ethanol (EtOH)
  - Milli-Q Water
  - Phosphoric Acid (H₃PO₄)

- **SOLUTION PREPARATION**

  **Mobile Phase: 0.1% Phosphoric Acid in Water**
  Solution was prepared by adding 1 mL of phosphoric acid to ~800 mL of milli-Q water in a 1000 mL volumetric flask and mixing well. The solution was then diluted to volume with milli-Q water, mixed well and transferred to a 1 L mobile phase container.

  **Diluent: 60:40 ACN-Water**
  Diluent was prepared by adding 300 mL of acetonitrile and 200 mL of milli-Q water into a 500 mL volumetric flask and mixing well.

  **70% Ethanol Solution**
  Solution was prepared by adding 175 mL of ethanol and 75 mL of milli-Q water into a 250 mL volumetric flask and mixing well.
• **STANDARD PREPARATION**

Acetoxyvalerenic Acid Stock Standard Solution
Approximately 3.0 mg of Acetoxyvalerenic Acid was weighed into a 10 mL volumetric flask. 10 mL of diluent was added and the solution sonicated for 15 minutes. Calibration standards were prepared from this stock solution.

Hydroxyvalerenic Acid Stock Standard Solution
Approximately 3.0 mg of Hydroxyvalerenic Acid was weighed into a 10 mL volumetric flask. 10 mL of diluent was added and the solution sonicated for 15 minutes. Calibration standards were prepared from this stock solution.

Valerenic Acid Stock Standard Solution
Approximately 3.0 mg of Valerenic Acid was weighed into a 10 mL volumetric flask. 10 mL of diluent was added and the solution sonicated for 15 minutes. Calibration standards were prepared from this stock solution.

• **SAMPLE PREPARATION**

Customer Sample CDXA-11-0596
Sample was prepared by weighing approximately 5.0 g of sample into a 100 mL volumetric flask. Flask was brought to volume with 70% ethanol solution and stirred for 1 hour at room temperature. The extract was then filtered using a 12.5 cm Whatman Qualitative #1 filter. The sample was filtered through a 0.45 μm PTFE filter into an HPLC vial for analysis.

Customer Sample CDXA-11-0597
Sample was prepared by weighing approximately 12.5 g of sample. 250 mL of water was added to the sample and the roots were liquefied by blending for 2 minutes. The solution was stirred for 1 hour at room temperature. The extract was then filtered using a 12.5 cm Whatman Qualitative #1 filter and centrifuged for 10 minutes at 3700 rpm. The sample was filtered through a 0.45 μm PTFE filter into an HPLC vial for analysis.
**INSTRUMENT PARAMETERS**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Agilent 1100 Series HPLC System</th>
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<tbody>
<tr>
<td>Detection</td>
<td>UV-Vis</td>
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<tr>
<td>Mobile Phase A</td>
<td>0.1% Phosphoric Acid</td>
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<tr>
<td>Mobile Phase B</td>
<td>Acetonitrile</td>
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**Gradient Program**

<table>
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<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
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<tbody>
<tr>
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<td>25</td>
</tr>
<tr>
<td>1.0</td>
<td>75</td>
<td>25</td>
</tr>
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<td>10.0</td>
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<td>25</td>
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<td>18.0</td>
<td>75</td>
<td>25</td>
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<table>
<thead>
<tr>
<th>Column</th>
<th>Phenomenex Luna C18(2)-HST 100 x 2.0 mm, 2.5μ</th>
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</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>0.500 mL/min</td>
</tr>
<tr>
<td>UV Detection</td>
<td>210 nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>3 μL</td>
</tr>
<tr>
<td>Temperature</td>
<td>50 °C</td>
</tr>
</tbody>
</table>
DATA

* FIGURES

Figure 1: Acetoxyvalerene Acid Stock Standard (UV Chromatogram)

Figure 2: Hydroxyvalerene Acid Stock Standard (UV Chromatogram)

Figure 3: Valerenic Acid Stock Standard (UV Chromatogram)

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DATA

• FIGURES

Figure 1: Acetoxylavulenic Acid Stock Standard (UV Chromatogram)

Figure 2: Hydroxyvalerenic Acid Stock Standard (UV Chromatogram)

Figure 3: Valerenic Acid Stock Standard (UV Chromatogram)

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REFERENCES

ChromaDex SOP "Routine Laboratory Calculations"
Analytical Method: S9.1-CD-3.0-000207 "Valerenic Acids in Valerian by HPLC."

<table>
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<tr>
<th>Laboratory Notebook</th>
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<tbody>
<tr>
<td>249</td>
<td>5-8</td>
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REVISION HISTORY

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