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Subject: FW: Embargoed paper
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From: "Wyszumiala, Edward" <ewyszumail@nsf.org>
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1,3-Dimethylamylamine (DMAA) in supplements and geranium plants/products: natural or synthetic?

Ying Zhang, Ross M. Woods and Daniel W. Armstrong*

1,3-Dimethylamylamine (DMAA) is a stimulant existing in various pre-workout supplements and often labelled as part of geranium plants. The safety and origin of DMAA in these supplements is the subject of intense debate. In this study, the enantiomeric and diastereomeric ratios of two different known synthetic DMAA compounds, as well as the total concentrations of DMAA and its stereoisomeric ratios in 13 different supplements, were determined by gas chromatography. The stereoisomeric ratios of DMAA in the synthetic standards and in all the commercial supplements were indistinguishable. Eight different geranium extracts of different geographical origins (China and the Middle East) were examined for the presence of DMAA by high performance liquid chromatography coupled with fluorescence detector (HPLC-Fl) and high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Trace amounts of DMAA were detected in only two geranium products with concentrations lower than 10 parts per million (w/w). Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: DMAA; GC analysis; HPLC analysis; Synthetic versus natural; Geranium oil

Introduction

1,3-Dimethylamylamine (DMAA), also known as 1,3-dimethylpentylamine, methylhexanamine and 2-amino-4-methylhexane, was first named 'Fortan' and introduced by Eli Lilly & Co., as a vasoconstrictor in the 1940s. After decades of relative obscurity, it was trademarked as geranamine and brought to the sports market as a dietary ingredient in various pre-workout supplements. This was possible because it was reported as a natural product extracted from geranium (Pelargonium graveolens) in a little known paper published in the Journal of Guizhou Institute of Technology in 1996.

In 2009, DMAA was added to the 2010 prohibited list by World Anti-Doping Agency (WADA) since it is a stimulant. In 2010 and 2011, some athletes were disqualified or stripped of their awards in various sporting events when DMAA was detected in post-event drug tests. Also, a few cases showed that DMAA might have serious side effects. In December 2010, the Journal of the New Zealand Medical Association reported that a 21-year-old man suffered a serious haemorrhage after taking DMAA containing pills subsequent to having an alcoholic drink. In December 2011, it was reported that the deaths of two US soldiers were suspected to be related to the use of DMAA containing supplements. In 2011, the American Herbal Products Association (AHPA) declared that supplement manufacturers should not label the stimulant DMAA as geranium oil or as any part of the geranium plant. This statement was supported by the United Natural Products Alliance (UNPA) in January 2012.

The safety of DMAA in supplements is the subject of intense debate. Since DMAA was considered by some to be a naturally occurring component of the geranium plant, products/supplements containing geranium-based entities avoided regulation by the Food and Drug Administration (FDA). However papers published in 1951 and 1960, as well as the National Cancer Institute (NCI) in vivo screening data of synthetic DMAA1, have shown there may be potential side effects and toxicity. One question is whether the DMAA in supplements is from geranium parts and extracts, or if it is a synthetic product. Other debated questions are whether DMAA should be regulated or even be allowed in supplements. The paper which was used as the only reference for introducing DMAA to the sporting world has been criticized due to its interpretation errors and lack of convincing evidence: (1) It mislabelled DMAA as 2-hexanamide, 4-methyl-; (2) There were three compounds listed in Table 1 of the paper as '28' = tricylene, '29' = 2-heptanamine, 5-methyl- and '30' = 2-hexanamide, 4-methyl- (mislabelled DMAA) between the two compounds 27 and 31. However, only one peak appeared between peak 27 and 31 in the gas chromatography-mass spectrometry (GC-MS) chromatogram. (3) In Table 1 of the paper, the concentrations of compound 27 and 31 were listed as 2.07% and 0.29%, respectively. The single peak that appeared between these two peaks in the GC-MS chromatogram obviously had a smaller peak area than these two peaks. However, the concentration of DMAA was reported as 0.66% which was larger than that of compound 31. (4) No standard was used to confirm the retention time and MS spectrum of DMAA. In December 2011, the National Measurement Institution of Australia published a short communication in Drug Testing and Analysis and asserted that geranium oils do not contain DMAA and the supplement products labelled containing geranium oil but which contain DMAA can only...
arise from the addition of synthetic material. Also, another researcher in National Science Foundation (NSF) Internationals failed to extract DMAA from several geranium essential oils available on the market. Despite this criticism, USPlabs insisted that DMAA in its products, Jack3d and OxyElite, was from geranium.

One important aspect of DMAA that has not been considered is that it is a chiral compound with two stereogenic centres (Figure 1). Hence the name DMAA does not refer to a single compound, but to a potential mixture of four stereoisomeric compounds (two pairs of enantiomers, with S,S- and R,R- configuration and R,S- and S,R- configuration, respectively). The enantiomeric pairs of synthetic DMAA must be racemic unless they result from an asymmetric process. Further, they will have a diastereomeric ratio characteristic of the synthetic process. Conversely natural plant-derived chiral compounds are usually enantiomerically enriched, often to a high degree. If diastereomers are present, they also would have a distinct, characteristic ratio.

In this study we determine the enantiomeric and diastereomeric ratios of two synthetic DMAA standards from different commercial sources. Subsequently, the total concentrations of DMAA and its stereoisomeric ratios were determined in 13 different supplements. Finally, eight different geranium extracts of different geographical origins (China and the Middle East) were examined for the presence of DMAA.

### Experimental

#### Materials

The supplement products were purchased from GNC (Pittsburgh, PA, USA), bodybuilding (Meridian, ID, USA), and Amazon (Seattle, WA, USA). 1,3-Dimethylpentylamine standard (free amine), pentafluoropropionic anhydride (PFPA), 2-aminopentane, dansyl chloride and trifluoroacetic acid were purchased from Sigma-Aldrich (Milwaukee, WI, USA). 1,3-Dimethylpentylamine hydrochloride was purchased from ChromaDex (Irvine, CA, USA). Sodium carbonate was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Anhydrous magnesium sulfate was purchased from EM Science (Gibbstown, NJ, USA). High performance liquid chromatography (HPLC) grade heptane, acetone, acetonitrile and dichloromethane were purchased from EMD Chemicals (Gibbstown, NJ, USA). Water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). The geranium oil extracts are listed in Table 1.

#### Sample preparation for GC analysis

1. Standard solutions: 10 mg of DMAA standard and 10 mg of internal standard (2-aminopentane) were dissolved in 0.5 ml of dichloromethane. 0.5 ml PFPA was added to the vial and the vial was sealed with a silicone rubber insert. The solution was heated for 30 min at 50°C. Then the solvent and residual PFPA were removed at room temperature under reduced pressure. The derivatized DMAA and internal standard were transferred to a 10-ml volumetric flask and diluted to 10 ml with heptane. The stock solution was diluted to a series of solutions with concentrations of 0.8, 0.6, 0.4, 0.2 mg/ml. (The concentrations refer to DMAA.) The calibration curves of DMAA and the internal standard were available in supporting materials. The response factor of DMAA to internal standard was calculated with the vials.

2. Supplements: 200 mg of supplement powder was dissolved in 1 ml water. 5 mg of internal standard was spiked into the solution. The pH was adjusted to 9–10 with sodium carbonate. One ml dichloromethane was added to the solution and vortexed. The whole solution was filtered with a syringe filter and the organic layer was collected and dried with magnesium sulfate. The dichloromethane solution was transferred to a 3 ml screw-top vial to which 0.5 ml PFPA was added and the vial sealed by cap with silicone rubber insert. The solution was heated for 30 min at 50°C. Then the solvent and residual PFPA were removed at room temperature under reduced pressure. The sample was diluted with heptane and ready for GC injection.

#### Sample preparation for HPLC analysis

1. DMAA was reacted with dansyl chloride at 1:3 stochiometry in acetone. 3M sodium carbonate was added to achieve a working concentration of 1M. Samples were protected from light and stirred at room temperature for 1 h. Dansyl-DMAA was extracted into 2 x 1 ml of dichloromethane. Organic layers were

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**Table 1. Geranium oils tested**

<table>
<thead>
<tr>
<th>No.</th>
<th>Oil analyzed</th>
<th>Manufacturer</th>
<th>Origin</th>
<th>Extraction method</th>
<th>Part of plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Geranium essential oil</td>
<td>Starwest Botanicals, Inc. (Rancho Cordova, CA, USA)</td>
<td>China</td>
<td>Steam distillation</td>
<td>Leaves and branches</td>
</tr>
<tr>
<td>2</td>
<td>Geranium essential oil</td>
<td>Earth Solutions (Atlanta, GA, USA)</td>
<td>China</td>
<td>Steam distillation</td>
<td>Flowers and stems</td>
</tr>
<tr>
<td>3</td>
<td>Geranium essential oil</td>
<td>Aura Cacial Urbana, IA, USA</td>
<td>China</td>
<td>Steam distillation</td>
<td>Leaves and flowering branchlets</td>
</tr>
<tr>
<td>4</td>
<td>SOMA Geranium oil</td>
<td>Dreaming Earth Botanicals (Asheville, NC, USA)</td>
<td>China</td>
<td>Steam distillation</td>
<td>Leaves and stems</td>
</tr>
<tr>
<td>5</td>
<td>Oshadhi Geranium select</td>
<td>Ayus Gmgh (Bühl, Baden-Württemberg, Germany)</td>
<td>Egypt</td>
<td>Steam distillation</td>
<td>Leaves</td>
</tr>
<tr>
<td>6</td>
<td>Geranium essential oil</td>
<td>Lotus Brands, Inc. (Twin Lakes, WI, USA)</td>
<td>Egypt</td>
<td>Steam distillation</td>
<td>Not labeled</td>
</tr>
<tr>
<td>7</td>
<td>Nature's Alchemy Geranium essential oil</td>
<td>Lotus Brands, Inc. (Twin Lakes, WI, USA)</td>
<td>Egypt</td>
<td>Steam distillation</td>
<td>Not labeled</td>
</tr>
<tr>
<td>8</td>
<td>Geranium essential oil</td>
<td>Now Foods (Bloomingdale, IL, USA)</td>
<td>Egypt</td>
<td>Steam distillation</td>
<td>Fresh plant</td>
</tr>
</tbody>
</table>

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**Figure 1.** The structure of 1,3-dimethylamylamine (DMAA).
DMAA in supplements: synthetic, not natural

combined and washed with 2 × 3 mL water and dried over magnesium sulfate. Solvents were removed at room temperature under reduced pressure and samples were diluted with acetonitrile for HPLC analysis. A series of solutions with concentrations of 100, 250, 500, 1000, and 2000 μg/L was prepared for the calibration curve. (The concentrations refer to DMAA.) The calibration curve was available in the supporting materials.

2. For geranium oils, the dansylation procedure was the same as above with 200 mg of geranium oil and 40 mg of dansyl chloride.

**GC method**

An Agilent model 6890N network gas chromatograph system was used. Helium was used as the carrier gas with a flow rate of 1 mL/min. The injection volume was 1 μL. The split ratio was 1:100 at the injector. Detection was achieved with an FID detector. The injector and detector temperatures were 250 °C. An Astec ChiralDex G-DM column (30 m × 0.25 mm i.d. × 0.20 μm) was used for all the GC separations. Determination of DMAA diastereomeric ratios and quantification of DMAA content in supplements were operated at 90 °C isothermally. 2-Aminopentane was used as internal standard for quantification of DMAA content in supplements. The enantiomeric excess of the DMAA in the standards and the supplements were determined at 30 °C isothermally.

**HPLC coupled with fluorescence detector (HPLC-FI) method**

In the HPLC-FI method, Shimadzu SIL-20AC autosampler, LC-20AD pump, RF-20Axs fluorescence detector and an Astec C18 column (25 cm × 4.6 mm) were used. Binary solvents were used for elution. Solvent A consisted of 100% acetonitrile and solvent B consisted of an aqueous solution with 0.1% trifluoroacetic acid. In each run, the mobile phase was isocratic. In different runs, the retention times of the analytes were adjusted by changing the ratio of the two solvents in the isocratic mobile phase. Total flow rate was 1 mL/min. The excitation wavelength and the emission wavelength for the fluorescence detector were set as 350 nm and 500 nm, respectively. The injection volume was 5 μL. The validation of this method is given in the supporting materials.

**HPLC-MS method**

In the HPLC-MS method, Thermo Finnigan Surveyor autosampler, MS pump, Thermo LQ linear ion trap mass spectrometer and an Astec C18 column (25 cm × 4.6 mm) were used. HPLC conditions were the same as described above. Total flow rate for HPLC was 1 mL/min. A splitter was used to control the flow entering MS, which was 0.3 mL/min. The mass spectra data were recorded in a positive mode of electrospray ionization for selected ion m/z 349. Capillary voltage and spray voltage were set at -7 V and 4.7 kV, respectively. When product ion scan experiments were conducted, the normalized collision energy was 30 (arbitrary units) while helium was used as the collision gas. The injection volume was 5 μL. The HPLC-MS chromatograms were available in the supporting materials.

**Results and discussion**

**GC analysis of DMAA in supplements**

The diastereomeric ratios of the synthetic DMAA standards from Sigma-Aldrich and ChromaDex were 1.22 ± 0.06 and 1.42 ± 0.09, respectively (Figure 2). As expected, both were racemic pairs of enantiomers (Figure 3A). The concentrations (weight %) and diastereomeric ratios of DMAA in 13 commercial supplements are given in Table 2. The total concentrations of DMAA varied widely in the supplements, from ~0.1% to ~11%. All diastereomeric ratios were in the same range as the two synthetic DMAA compounds, *vide supra*. Furthermore, the enantiomeric compositions of the DMAA in all 13 supplements were racemic (Figure 3B). Thus, the stereoisomeric compositions of DMAA in the synthetic standards and in all the commercial supplements are indistinguishable. The concentrations of DMAA in most of the supplements were fairly high. In general, the concentrations of molecules with low molecular weight in botanicals and their extracts are not that high,²⁵,²⁶ and therefore their concentrations in commercial products containing a small proportion of the botanicals/extracts would be even lower. Consequently, the level (concentration), nature (stereoisomeric composition) and existence of DMAA in geranium plants/extracts are particularly germane to the ongoing debate.

**HPLC analysis of geranium oils**

To determine if geranium oil contains DMAA, a detection method with high sensitivity is preferred. HPLC-FI was used in this study. By using fluorescence detection, the limit of detection (LOD) of DMAA standard was 1 μg/L when it was eluted at about 7 min and 25 μg/L when it was eluted at about 70 min, depending on the isocratic elution condition used (Experimental section). It should be noted that in the discussion of all HPLC results, the concentrations of DMAA referred to is that of neat DMAA and not the concentration of the derivatization product, dansyl DMAA. Eight geranium...
oils purchased from different manufacturers were extracted by steam distillation method which is the same as the method used by Zang et al. All eight geranium oils were fully derivatized with dansyl chloride and analyzed by HPLC-FI. Among the eight geranium oil samples, two geranium oils, which were manufactured by Now Foods and Earth Solutions, showed two peaks at retention times which were the same as that of the diastereomers of the dansyl DMAA standards. As shown in Figure 4, a few co-eluted components in the geranium oil were separated from the two peaks when the percentage of acetonitrile in the mobile phase was altered.

Table 2. The diastereomeric ratio and concentration of DMAA in supplements

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Manufacturer</th>
<th>Diastereomeric Ratio</th>
<th>% DMAA Dry weight</th>
<th>DMAA per serving (mg)</th>
<th>Stated DMAA per serving (mg)</th>
<th>Labelling DMAA as</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1,3-DIMETHYLAMYLAMINE</td>
<td>Primaforce</td>
<td>1.23</td>
<td>3.7 ± 0.4</td>
<td>17 ± 2</td>
<td>20</td>
<td>1,3-dimethylamylamine</td>
</tr>
<tr>
<td>2 Speed V2 diet pills</td>
<td>LG Science</td>
<td>1.28</td>
<td>0.20 ± 0.06</td>
<td>1.2 ± 0.4</td>
<td>*</td>
<td>geranium oil extract</td>
</tr>
<tr>
<td>3 ADRALIN dietary supplement</td>
<td>CTD Labs</td>
<td>1.31</td>
<td>2.1 ± 0.3</td>
<td>34 ± 4</td>
<td>*</td>
<td>1,3-dimethylpentylamine</td>
</tr>
<tr>
<td>4 RIPPED JUICE</td>
<td>BETANCOURT NUTRITION</td>
<td>1.34</td>
<td>11.2 ± 1.0</td>
<td>80 ± 7</td>
<td>*</td>
<td>geranium</td>
</tr>
<tr>
<td>5 OxyELITE Pro</td>
<td>USPlabs</td>
<td>1.36</td>
<td>10.2 ± 1.7</td>
<td>31 ± 5</td>
<td>*</td>
<td>1,3-dimethylpentylamine hydrochloride</td>
</tr>
<tr>
<td>6 Jack3d</td>
<td>USPlabs</td>
<td>1.43</td>
<td>2.6 ± 0.5</td>
<td>142 ± 25</td>
<td>*</td>
<td>geranium stem</td>
</tr>
<tr>
<td>7 FlashOver</td>
<td>Omega Sports</td>
<td>1.32</td>
<td>2.9 ± 0.5</td>
<td>285 ± 51</td>
<td>20</td>
<td>1,3-dimethylamylamine</td>
</tr>
<tr>
<td>8 OVERDOSE</td>
<td>NRGX LABS</td>
<td>1.27</td>
<td>0.11 ± 0.01</td>
<td>217 ± 26</td>
<td>*</td>
<td>geranium stem</td>
</tr>
<tr>
<td>9 PWR</td>
<td>Isatori, LLC</td>
<td>1.28</td>
<td>0.33 ± 0.09</td>
<td>16 ± 4</td>
<td>*</td>
<td>1,3-dimethylpentylamine</td>
</tr>
<tr>
<td>10 1MR</td>
<td>BPI</td>
<td>1.31</td>
<td>1.1 ± 0.1</td>
<td>85 ± 9</td>
<td>*</td>
<td>1,3-dimethylamylamine</td>
</tr>
<tr>
<td>11 STIM-FORCE</td>
<td>LABRADA NUTRITION</td>
<td>1.31</td>
<td>0.72 ± 0.04</td>
<td>27 ± 1</td>
<td>*</td>
<td>1,3-dimethylpentylamine hydrochloride</td>
</tr>
<tr>
<td>12 HEMO RAGE</td>
<td>Nutrex research, Inc.</td>
<td>1.35</td>
<td>1.03 ± 0.04</td>
<td>33 ± 1</td>
<td>*</td>
<td>1,3-dimethylpentylamine</td>
</tr>
<tr>
<td>13 HYDROXYSTIM</td>
<td>MuscleTech</td>
<td>1.25</td>
<td>1.9 ± 0.2</td>
<td>10 ± 1</td>
<td>177</td>
<td>geranium extract</td>
</tr>
</tbody>
</table>

* The amount of DMAA per serving in the supplement was not stated.

Figure 4. The fluorescence chromatograms of dansyl DMAA standard (the black lines, concentration: 10 mg/L) and dansylated Now Foods geranium essential oil (the red lines) with 4 different mobile phases: A. 80%ACN + 20%H2O (containing 0.1% TFA), retention times of the DMAA diastereomers were 6.72 min and 6.85 min; B. 60%ACN + 40%H2O (containing 0.1% TFA), retention times of the DMAA diastereomers were 15.45 min and 15.90 min; C. 50%ACN + 50%H2O (containing 0.1% TFA), retention times of the DMAA diastereomers were 29.01 min and 29.90 min; D. 40%ACN + 60%H2O (containing 0.1% TFA), retention times of the DMAA diastereomers were 70.35 min and 73.03 min. The diastereomeric ratios were 1.4 and 0.8 for the ChromDex standard and the Now Foods essential geranium oil, respectively.
was decreased. When the retention time was increased to 70 min, the two peaks in the Now Foods geranium oil showed a different ratio compared to the diastereomeric ratio of the dansyl DMAA standards. As seen in Figure 4D, the first peak was larger than the second one in the Now Foods geranium oil sample, which was opposite to the observation for the dansyl DMAA standard. It should be noted that the retention order of the two diastereomers of DMAA in the HPLC chromatograms was the opposite of that for the GC separations shown in Figure 2. A similar result was obtained for the Earth Solutions geranium oil.

HPLC-MS method was used to further confirm if these two peaks in the geranium oils were dansyl DMAA. All the eight dansylated geranium oils were analyzed by HPLC-MS with a mobile phase consisting of 40% acetonitrile and 60% H$_2$O (containing 0.1% TFA). Again, only the Now Foods and Earth Solutions geranium oils showed detectable signals at the retention times for dansyl DMAA standard in the selected ion mode (m/z 349). These peaks in the two geranium oils had the same fragmentation in their tandem MS spectra (Figure 5B), as the dansyl DMAA standard (Figure 5A). (The HPLC-MS chromatograms of the DMAA standard and the geranium oil sample were available in supporting materials.) Therefore, it appears that the two geranium oils, manufactured by Now Foods and Earth Solutions, contained a very small amount of DMAA.

The dansyl derivatives of three isomers of DMAA, 1,4-dimethylpentylamine, 2-aminoheptane and heptylamine, were also analyzed by HPLC-FI. As shown in Figure 6, dansyl-1,
4-dimethylpentylamine and dansyl-2-aminoheptane were eluted after the dansyl-DMAA diastereomers. The dansyl-heptylamine did not elute within 100 min when using the same HPLC method. Thus the two peaks in geranium oils which had the same retention times as the DMAA standard were not these isomers of DMAA.

Since it was confirmed that these two geranium oils contained DMAA, the concentrations of DMAA in these two geranium oils was quantified by using the calibration curve of the HPLC-FI method. There were 7 mg/kg and 3 mg/kg of DMAA in the DMAA, the concentrations of DMAA in these two geranium oils respectively.

Now Foods geranium oil and the Earth Solutions geranium oil, According to the GC and HPLC analyses in this study, it appears unlikely that the DMAA in supplements originates from natural sources such as geranium oils for four reasons: (1) the DMAA extracted from these supplement products have very similar diastereomeric ratios as the synthetic DMAA standards; (2) they are not racemic; (3) the DMAA detected in geranium oils have different diastereomeric ratio compared to the DMAA in the supplements; and (4) the very low concentrations of DMAA found in only two geranium oils could not account for the high levels of DMAA found in supplements.

Conclusions

According to the GC and HPLC analyses in this study, it appears unlikely that the DMAA in supplements originates from natural sources such as geranium oils for four reasons: (1) the DMAA extracted from these supplement products have very similar diastereomeric ratios as the synthetic DMAA standards; (2) they are all racemic; (3) the DMAA detected in geranium oils have different diastereomeric ratio compared to the DMAA in the supplements; and (4) the very low concentrations of DMAA found in only two geranium oils could not account for the high levels of DMAA found in supplements.

Acknowledgement

We thank Anthony Almada (GENr8, Inc., Dana Point, CA) for useful discussions especially in indicating early relevant references.

References