Exhibit 18

The highlighted portions of the attached exhibit illustrate the portions of the Zhang/Armstrong article that were altered from the unpublished version.
1,3-Dimethylamylamine (DMAA) in supplements and geranium products: natural or synthetic?

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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 commercial 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 mass spectrometry (HPLC-MS). No DMAA was detected in any of the eight geranium products with a limit of detection of 10 parts per billion (w/w). Copyright © 2012 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

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

Introduction

1,3-Dimethylamylamine (DMAA), also known as 1,3-dimethylpentylamine, methylhexaneamine and 2-amino-4-methylhexane, was first named 'Forthane' 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 the 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, the National Measurement Institute 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

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arise from the addition of synthetic material.121 Also, another researcher in National Science Foundation Internationals failed to detect DMAA from several geranium essential oils available on the market.141 Despite this criticism, USPlabs insisted that DMAA in its products, Jack3d and OxyElite, was from geranium.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.124 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), pentfluoropropionic 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 and sodium hydroxide were 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, methanol, hexane, diethyl ether, ethyl acetate 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. A eucalyptus essential oil (Now Foods, Bloomingdale, IL, USA) was purchased from GNC (Pittsburgh, PA, USA).

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 are available in supporting materials. The response factor of DMAA to internal standard was calculated from the calibration curves and equal to 1,02.

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. 1 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. Non-spiked sample: 2 g of geranium oil were weighed into a 50 ml vial. 10 ml of de-ionized water was added and

![Figure 1. The structure of 1,3-dimethylamylamine (DMAA).](image-url)
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followed by 1 mL of concentrated HCl solution. The sample was vortexed. The sample was then extracted with 2 - 10 mL hexane/diethyl ether (50/50). The organic layers were discarded. 1 mL of 50% NaOH solution was added to the sample and briefly vortexed. The sample was then extracted with 3 x 4 mL hexane/ethyl acetate (50/50). The organic portion was collected and evaporated to dryness.

2. Spiked sample: 100 parts per billion (w/w) DMAA hydrochloride standard was spiked into each geranium oil sample and extracted by using the above procedure.

3. For HPLC-electrospray ionization-linear ion trap (HPLC-ESI-LIT) analysis, the extracted residue was dissolved in 500 μL of methanol which contained 0.1% formic acid prior to injection.

4. For HPLC-electrospray ionization-triple quadrupole (HPLC-ESI-QQQ) analysis, the extracted residue was danylized prior to analysis. In the danylization procedure, the extracted residue was dissolved in 3 mL of 2:1 acetone:3M sodium carbonate. Then 25 μg of dansyl chloride was added and the reaction was stirred in darkness for 20 minutes at 60 C. 2 mL of dichloromethane was added and the organic layer was washed with 2 x 10 mL of de-ionized water. The solvent was removed under vacuum and the residue was dissolved in 1 mL of acetonitrile prior to injection.

5. Two blanks were prepared for each analysis method: 1) in one blank no essential oil was used, 2) in the other blank an equal amount of eucalyptus essential oil (Now Foods, Bloomingdale, IL, USA) was used instead of geranium oil. The blanks were prepared following the same procedures above for each analysis method.

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 x 0.25 mm i.d. x 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 30 C isothermally. 2-Aminopentane was used as internal standard in the quantification of DMAA in the supplements. The enantionic excess of the DMAA in the standards and the supplements were determined at 30 C isothermally.

HPLC-ESI-LIT method

In the HPLC-ESI-LIT method, a Thermo Finnigan Surveyor autosampler, a MS pump, a ThermoLXQ linear ion trap mass spectrometer and a LARIHC C6F-6 column (15 cm × 2.1 mm) (AZYP, Arlington, TX, USA) were used. The HPLC mobile phase consisted of 70% of acetonitrile and 30% of H2O containing 0.1% trifluoroacetic acid. The total flow rate was 0.2 mL/min. Capillary voltage and spray voltage were set at 25 V and 5 kV, respectively. The injection volume was 5 μL. The LOD of this method was 10 ppb.

HPLC-ESI-QQQ method

In the HPLC-ESI-QQQ method, a Shimadzu SIL-20AC autosampler, a LD-20AD pump, a LC/MS-8030 triple quadrupole mass spectrometer and a Kinetex 2.6u XB-C18 column (10 cm × 2.1 mm) were used.

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 enantionic 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 were 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, and therefore their concentrations in commercial products containing a small proportion of the botanicals/extracts would be even lower. Consequently, the level (concentration). Products containing a small proportion of the botanicals/extracts are particularly germane to the ongoing 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. In this study, two mass spectrometric methods were used for the detection of DMAA. One used an ESI-linear ion trap mass spectrometer (ESI-LIT-MS) and the other utilized an ESI-triple quadrupole mass spectrometer (ESI-QQQ-MS). When the underivatized extracted residues of geranium oils were directly injected to these mass spectrometers, the LOD was significantly reduced when the components in the sample. This column provided adequate retention of primary amines when using polar organic mobile phases. The retention time of DMAA in this column was about 8 min. (See Experimental and Supporting Information.) The LOD of this method was 50 ppb for underivatized DMAA in geranium oil. No DMAA was detected in any of the 8 geranium oil samples with this method.

Dansylation can change the polarity and increase the retention of DMAA in the reversed phase mode HPLC. Also, the dansylation can reduce the LOD of DMAA. Thus, the extracted residues of the geranium oils were dansylated prior to the HPLC-ESI-QQQ analysis (See Experimental). The LOD of this method was 10 ppb for DMAA in geranium oil. It should be noted that the LOD of DMAA refers to the concentration of neat DMAA in the geranium oils and not to the concentration of the derivatization product, dansyl DMAA. Again, no DMAA was detected in any of the 8 geranium oil samples.

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 three reasons: (1) the DMAA extracted from these supplement products had diastereomeric ratios that were indistinguishable from the synthetic DMAA standards; (2) they are all racemic; (3) no DMAA was detected at a level of ≥10 ppb in any of the 8 geranium oil samples.

Acknowledgement

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Supporting information

Supporting information may be found in the online version of this article.

Conflict of interest statement

The corresponding author, D. W. A., served as an expert witness in 2011 for the case: DeRosier v. USPlabs.

References

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