Exhibit 16

Brandi Johnson

From: Mahmoud A. Elsohly [melsohly@olemiss.edu]
Sent: Wednesday, May 23, 2012 2:15 PM
To: elsohly@elsohly.com
Subject: FW: Embargoed paper
Attachments: image001.jpg; ATT00001..htm; DTA-EmbargoedMay30 2012-00.01EDT-DMAA.pdf; ATT00002..htm

Mahmoud A. ElSohly

Mahmoud A. ElSohly, Ph.D.
Research Professor, Project Director
The University of Mississippi
National Center for Natural Products Research
a division of The Research Institute of Pharmaceutical Sciences
135 Coy Waller Laboratory Complex
University, MS 38677
☎662.915.5928 phone
🖷662.915.5587 fax
melsohly@olemiss.edu

From: Amy Eichner, Ph.D. [mailto:AEichner@usada.org]
Sent: Wednesday, May 23, 2012 7:56 AM
To: Mahmoud A. Elsohly; Ikhlas Khan
Subject: Embargoed paper

Have you seen this? What are your thoughts?

Amy Eichner
Sent from my iPhone

Begin forwarded message:

> **From:** "Bestervelt, Lori" <Bestervelt@nsf.org>
> **Date:** 23 May 2012 5:24:59 AM MDT
> **To:** "Amy Eichner, Ph.D." <AEichner@usada.org>, Patty Deuster <patricia.deuster@usuhs.edu>
> **Subject: Fwd: [Phys] Request from journalist**
>
> Sent from my iPhone
>
> Begin forwarded message:

From: "Wyszumiala, Edward" <ewyszumail@nsf.org>
To: "Bestervelt, Lori" <Bestervelt@nsf.org>, "Travis, John" <travis@nsf.org>
Subject: Fw: [Phys] Request from journalist

Sent from my Verizon Wireless BlackBerry

USADA

U.S. Anti-Doping Agency

***Preserving** the integrity of competition*
***Inspiring** true sport*
***Protecting** the rights of athletes*

Amy Eichner, Ph.D.
Special Advisor on Drugs and Supplements

5555 Tech Center Drive, Suite 200
Colorado Springs, CO 80919

719-785-2043 Phone
866-601-2632 Toll Free
719-785-2001 Fax

aeichner@usada.org

Stay Connected: www.usada.org | Facebook | Twitter

From: Elaine WATSON <Elaine.WATSON@wrbm.com>
Date: Tue, 22 May 2012 18:24:52 +0200
To: <ewyszumail@nsf.org>
Subject: FW: [Phys] Request from journalist

Here's the embargoed paper attached

Elaine Watson
Correspondent
FoodNavigator-USA.com and NutraIngredients-USA.com
http://www.FoodNavigator-USA.com
http://www.NutraIngredients-USA.com
Tel: 1 937 388 0636
Email: elaine.watson@wrbm.com

physically or otherwise and notify the sender as soon as possible.

We reserve the right to read any e-mail or attachment entering or leaving our systems from any source without prior notice.

William Reed Business Media Ltd. Registered office: Broadfield Park, Crawley RH11 9RT, England. Registered in England No. 2883992.

William Reed Business Media SAS. Registered Office: Le Belem, 355, rue Vendémiaire, 34000 Montpellier, France. Registered in France – RCS Montpellier 429.315.971.

Research article

Received: 20 March 2012 Revised: 6 April 2012 Accepted: 9 April 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/dta.1368

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-FI) 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 part 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, methylhexaneamine and 2-amino-4-methylhexane, was first named 'Forthane' and introduced by Eli Lilly & Co., as a vasoconstrictor in the 1940s.[1–3] 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.[4] 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.[5]

In 2009, DMAA was added to the 2010 prohibited list by World Anti-Doping Agency (WADA) since it is a stimulant.[6] 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.[7–9] 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.[10] 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.[11,12] 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.[4] This statement was supported by the United Natural Products Alliance (UNPA) in January 2012.[13]

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[14] and 1960[14–16] as well as the National Cancer Institute (NCI) *in vivo* screening data of synthetic DMAA[17] 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.[18,19] Other debated questions are whether DMAA should be regulated or even be allowed in supplements.[4,20] The paper[5] 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-.[4,21] (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

* *Correspondence to: Daniel Armstrong, Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019. E-mail: sec4dwa@uta.edu*

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019, USA

Table 1. Geranium oils tested

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

arise from the addition of synthetic material.[22] Also, another researcher in National Science Foundation (NSF) Internationals failed to extract DMAA from several geranium essential oils available on the market.[4] Despite this criticism, USPlabs insisted that DMAA in its products, Jack3d and OxyElite, was from geranium.[23]

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.[24] 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, PN, USA), bodybuilding (Meridian, ID, USA), and Amazon (Seattle, WA, USA). 1,3-Dimethylpentylamine standard (free amine), pentafluoropropionic anhydride (PFPA), 2-aminopentane, dansyl chloride and trifluroacetic 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.

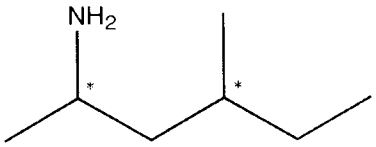


Figure 1. The structure of 1,3-dimethylamylamine (DMAA).

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 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. 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 × 1 ml of dichloromethane. Organic layers were

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 1mL/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 distereomeric ratios and quantification of DMAA content in supplements were operated at 90 °C isothermally. 2-Aminopentane was used as internal standard in the quantification of DMAA in the 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, Shimazhu SIL-20AC autosampler, LD-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 LXQ linear ion trap mass spectrometer and an Astec C18 column (25cm × 4.6mm) 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 -7V and 4.7kV, 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

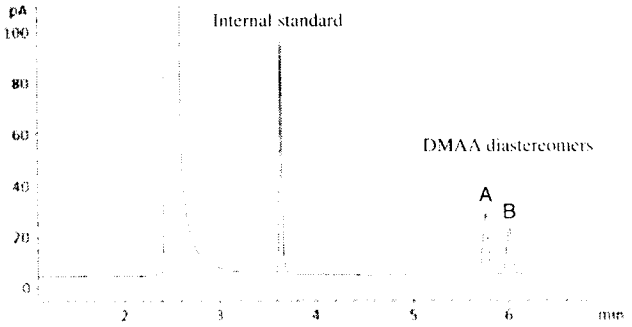


Figure 2. GC chromatogram of N-pentafluoropropionyl derivatives of DMAA standard (from Sigma-Aldrich) and internal standard at 90 °C. Diastereomeric ratio = Peak Area A/ Peak Area B. The retention times of the internal standard, DMAA diastereomer A and B were 3.62 min, 5.78 min, and 6.20 min, respectively.

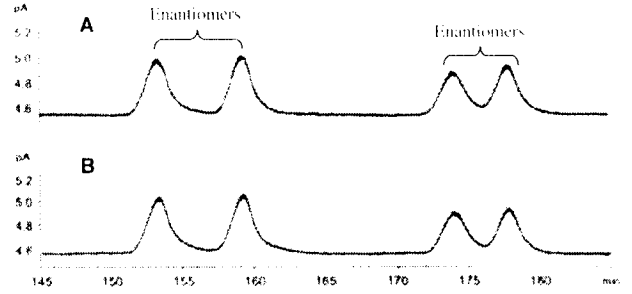


Figure 3. GC chromatograms of N-pentafluoropropionyl DMAA enantiomers at 30 °C. A)Synthetic DMAA, B) DMAA in the supplement manufactured by Primaforce. DMAA in all the other supplements had identical chromatograms as B).The retention times for the four enantiomers were 153.49 min, 159.18 min, 174.11 min and 177.71 min, respectively.

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 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,[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 concentration of DMAA referred to is that of neat DMAA and not the concentration of the derivatization product, dansyl DMAA. Eight geranium

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

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

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

oils purchased from different manufacturers were extracted by steam distillation method which is the same as the method used by Zang *et al.*[5] All eight geranium oils were fully derivatized with dansyl chloride and analyzed by HLPC-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

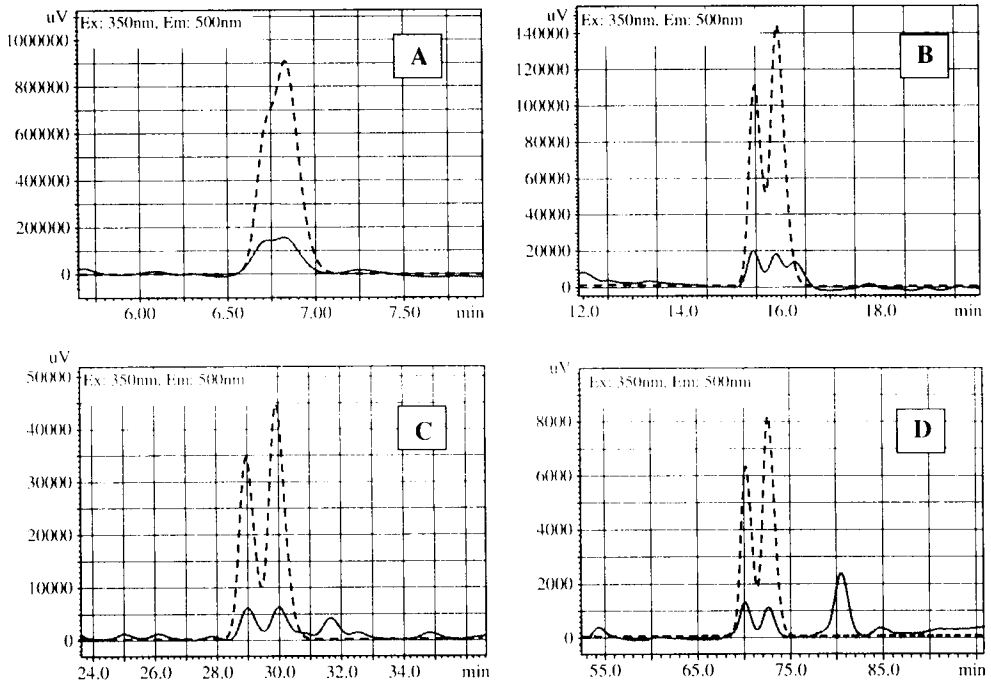


Figure 4. The fluorescence chromatograms of dansyl DMAA standard (the black lines, concentration: 10 mg/L) and dansylated Now Foods geranium essential oil (the red lines) with 4 different mobile phases: A. 80%ACN + 20%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 6.72 min and 6.85 min; B. 60%ACN + 40%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 15.45 min and 15.90 min; C. 50%ACN + 50%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 29.01 min and 29.90 min; D. 40%ACN + 60%H_2O(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_2O (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,

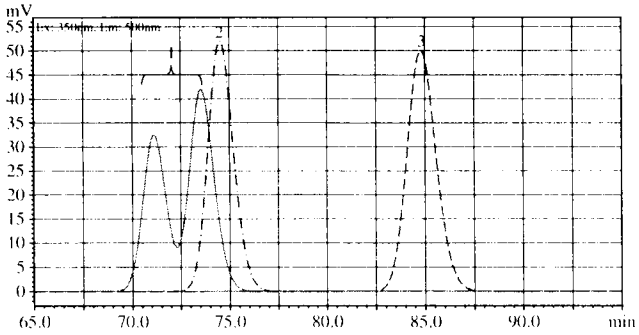


Figure 6. The fluorescence chromatograms of dansyl derivatives of DMAA and its isomers. 1. Diastereomers of dansyl-1,3-dimethylamylamine (dansyl-DMAA), 2. dansyl-1,4-dimethylpentylamine, 3. dansyl-2-aminoheptane. Mobile phase: 40%ACN + 60%H_2O(containing 0.1%TFA). Flow rate: 1 ml/min.

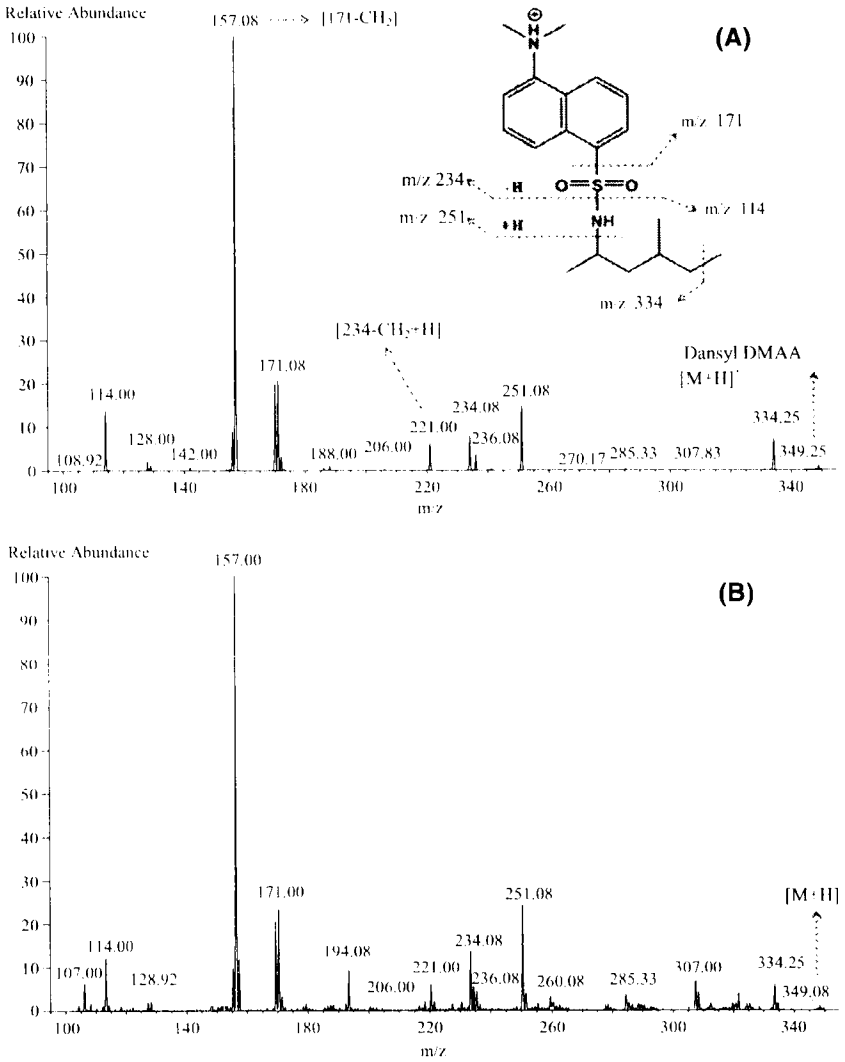


Figure 5. The tandem MS spectra of (A) dansyl DMAA standard (from ChromaDex) and (B) dansylated Now Foods geranium oil. Parent ion *m/z*: 349, width: 3.

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 Now Foods geranium oil and the Earth Solutions geranium oil, respectively.

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

[1] H.A. Shonle, E. Rohrmann. US Patent No 2350318, **1944**.
[2] E. Rohrmann, H.A. Shonle. Amino alkanes as pressor substances. *J. Am. Chem. Soc.* **1944**, *66*, 1516.
[3] Anonymous. New and nonofficial remedies: methylhexamine; forthane. *J. Am. Med. Assoc.* **1950**, *143*, 1156.
[4] Anonymous. What to do about DMAA? *Nutr. Bus. J.* **2012**, *17*, 1.
[5] P. Zang, J. Qing, Q. Lu. A study on the chemical constituents of geranium oil. *Guizhou Gongxueyuan Xuebao* **1996**, *25*, 82.
[6] World Anti-doping Agency. WADA 2010 Prohibited List. Available at: http://www.wada-ama.org/Documents/World Anti-Doping - Program/WADP-Prohibited-list/WADA Prohibited List 2010 EN. pdf [19 September 2009].
[7] NDTV. Now nine Aussie athletes test positive for methylhexaneamine. Available at: http://www.ndtv.com/article/commonwealth%20games/now-nine-aussie-athletes-test-positive-for-methylhexaneamine-61768&cp [23 October 2010].
[8] P. Cossins. Rui Costa and his brother test positive. Available at: http://www.cyclingnews.com/news/rui-costa-and-his-brother-test-positive [19 October 2010].
[9] BBC Sport. Second Nigerian tests positive at Commonwealth Games. Available at: http://news.bbc.co.uk/sport2/hi/commonwealth games/delhi_2010/9082481.stm [12 October 2010].
[10] P. Gee, S. Jackson, J. Easton. Another bitter pill: a case of toxicity from DMAA party pills. *N. Z. Med. J.* **2010**, *123*, 124.
[11] T.J. Tritten. Army probing connection between body building supplement, 2 deaths. Available at: http://www.stripes.com/news/army-probing-connection-between-body-building-supplement-2-deaths-1.163652 [15 December 2011].
[12] P. Chiaramonte. Soldier deaths during training prompt military probe into supplement use. Available at: http://www.foxnews.com/us/2012/02/02/soldier-deaths-during-training-sparks-military-probe-into-supplement-use/ [2 February 2012].
[13] E. Watson. UNPA: We agree with AHPA on DMAA labeling. Available at: http://www.nutraingredients-usa.com/Industry/UNPA-We-agree-with-AHPA-on-DMAA-labeling [10 January 2012].
[14] D.F. Marsh, A. Howard, D.A. Herring. The comparative pharmacology of the isomeric nitrogen-methyl-substituted heptylamines. *J. Pharmacol. Exp. Ther.* **1951**, *103*, 325.
[15] R.B. Stoughton, G. Deoreo, W. Clendenning. Effects of intradermal injection of vasopressors in normal and diseased human skin. *Arch. Dermatol.* **1960**, *82*, 400.
[16] D.T. Walz, T. Koppanyi, G.D. Maengwyn-Davies, M.L. Joyce. Isoproterenol vasomotor reversal by sympathomimetic amines. *J. Pharmacol. Exp. Ther.* **1960**, *129*, 200.
[17] NCI. In vivo screening data. Available at: http://dtp.nci.nih.gov/dtpstandard/servlet/InvivoScreen?testshortname=Tumor+LE+(ip)+in+06&searchlist=1106&searchtype=NSC [18 March 2012].
[18] S. Daniells. Health Canada: DMAA is not from geranium. Available at: http://www.nutraingredients-usa.com/Industry/Health-Canada-DMAA-is-not-from-geranium [24 August 2011].
[19] E. Adelson. Real Heat Wave Risk Posed by Fake "Geranium". Available at: http://www.thepostgame.com/features/201108/geranium-products-might-pose-serious-risk-athletes [15 August 2011].
[20] S. Starling. Food? Medicine? Neither? UK agencies "trying to get to bottom" of DMAA status. Available at: http://www.nutraingredients.com/Regulation/Food-Medicine-Neither-UK-agencies-trying-to-get-to-bottom-of-DMAA-status/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=Newsletter%2BDaily&c=gaa%2FSjqPjaAElOqUhpyCi%2BX4aUxc3sFA [23 February 2012].
[21] Anonymous. Geranium oil research. **2012**. Available at: http://dmaare-search.com/geranium-oil-research [18 March 2012].
[22] A. Lisi, N. Hasick, R. Kazlauskas, C. Goebel. Studies of methylhexaneamine in supplements and geranium oil. *Drug Test. Anal.* **2011**, *3*, 873.
[23] E. Watson. USPLabs: DMAA is from geranium oil - and critics are "uninformed". Available at: http://www.nutraingredients-usa.com/Regulation/USPLabs-DMAA-is-from-geranium-oil-and-critics-are-uninformed [5 January 2012].
[24] D.W. Armstrong, X. Wang, J. Lee, Y. Liu. Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco. *Chirality* **1999**, *11*, 82.
[25] T. Mroczek, K. Glowniak, A. Wlaszczyk. Simultaneous determination of N-oxides and free bases of pyrrolizidine alkaloids by cation-exchange solid-phase extraction and ion-pair high-performance liquid chromatography. *J. Chromatogr. A* **2002**, *949*, 249.
[26] L. Zhou, A.A. Hopkins, D.V. Huhman, L.W. Sumner. Efficient and sensitive method for quantitative analysis of alkaloids in hardinggrass (Phalaris aquatica L.). *J. Agric. Food Chem.* **2006**, *54*, 9287.

Exhibit 17

Research article

Received: 20 March 2012 Revised: 6 April 2012 Accepted: 9 April 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/dta.1368

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-FI) 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 part per million (*w/w*).

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.[1–3] 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.[4] 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.[5]

In 2009, DMAA was added to the 2010 prohibited list by World Anti-Doping Agency (WADA) since it is a stimulant.[6] 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.[7–9] 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.[10] 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.[11,12] 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.[4] This statement was supported by the United Natural Products Alliance (UNPA) in January 2012.[13]

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[14] and 1960[14–16] as well as the National Cancer Institute (NCI) *in vivo* screening data of synthetic DMAA[17] 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.[18,19] Other debated questions are whether DMAA should be regulated or even be allowed in supplements.[4,20] The paper[5] 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-.[4,21] (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

* *Correspondence to: Daniel Armstrong, Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019. E-mail: sec4dwa@uta.edu*

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019, USA

Table 1. Geranium oils tested

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

arise from the addition of synthetic material.[22] Also, another researcher in National Science Foundation (NSF) Internationals failed to extract DMAA from several geranium essential oils available on the market.[4] Despite this criticism, USPlabs insisted that DMAA in its products, Jack3d and OxyElite, was from geranium.[23]

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.[24] 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, PN, USA), bodybuilding (Meridian, ID, USA), and Amazon (Seattle, WA, USA). 1,3-Dimethylpentylamine standard (free amine), pentafluoropropionic anhydride (PFPA), 2-aminopentane, dansyl chloride and trifluroacetic 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.

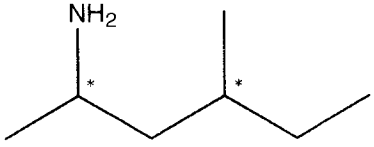


Figure 1. The structure of 1,3-dimethylamylamine (DMAA).

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 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. 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 × 1 ml of dichloromethane. Organic layers were

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 1mL/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 distereomeric ratios and quantification of DMAA content in supplements were operated at 90 C isothermally. 2-Aminopentane was used as internal standard in the quantification of DMAA in the 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, Shimazhu SIL-20AC autosampler, LD-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 LXQ linear ion trap mass spectrometer and an Astec C18 column (25cm × 4.6mm) 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 -7V and 4.7kV, 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

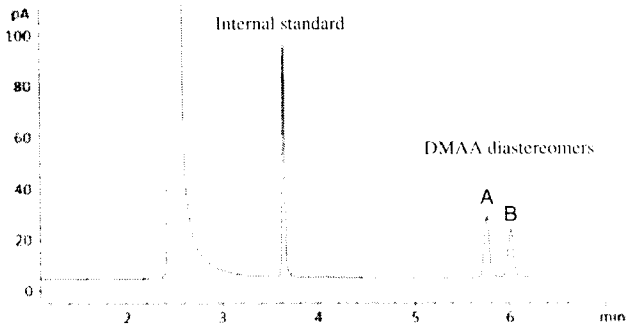


Figure 2. GC chromatogram of N-pentafluoropropionyl derivatives of DMAA standard (from Sigma-Aldrich) and internal standard at 90 C. Diastereomeric ratio = Peak Area A/ Peak Area B. The retention times of the internal standard, DMAA diastereomer A and B were 3.62 min, 5.78 min, and 6.20 min, respectively.

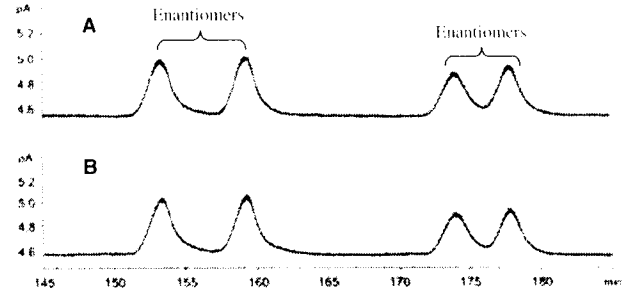


Figure 3. GC chromatograms of N-pentafluoropropionyl DMAA enantiomers at 30 C. A)Synthetic DMAA, B) DMAA in the supplement manufactured by Primaforce. DMAA in all the other supplements had identical chromatograms as B).The retention times for the four enantiomers were 153.49 min, 159.18 min, 174.11 min and 177.71 min, respectively.

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 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,[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 concentration of DMAA referred to is that of neat DMAA and not the concentration of the derivatization product, dansyl DMAA. Eight geranium

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

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

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

oils purchased from different manufacturers were extracted by steam distillation method which is the same as the method used by Zang *et al.*[5] All eight geranium oils were fully derivatized with dansyl chloride and analyzed by HLPC-Fl. 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

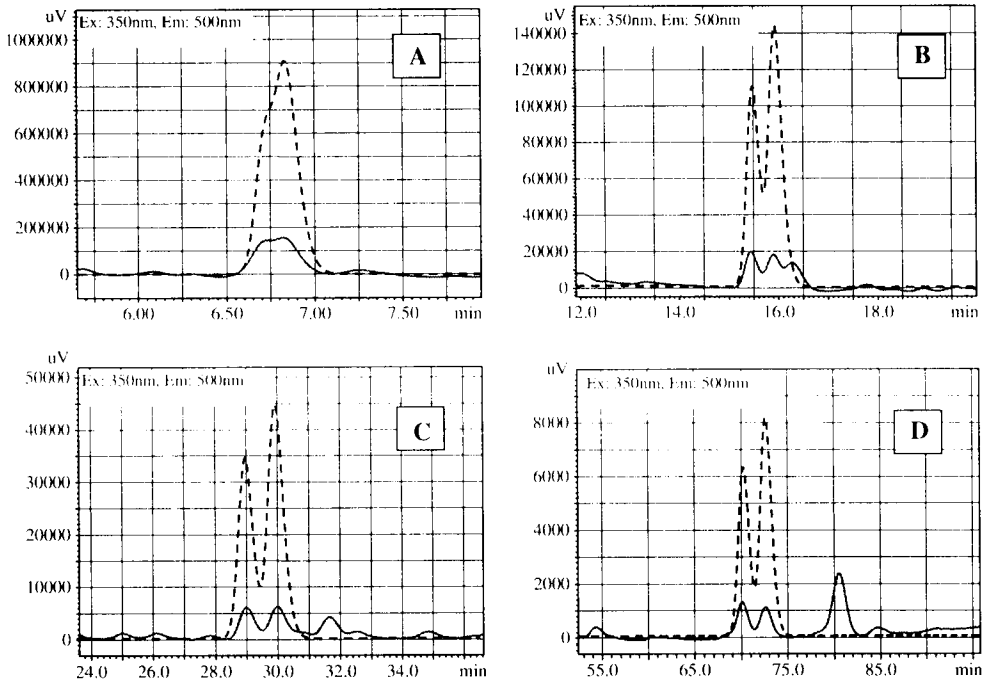


Figure 4. The fluorescence chromatograms of dansyl DMAA standard (the black lines, concentration: 10 mg/L) and dansylated Now Foods geranium essential oil (the red lines) with 4 different mobile phases: A. 80%ACN + 20%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 6.72 min and 6.85 min; B. 60%ACN + 40%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 15.45 min and 15.90 min; C. 50%ACN + 50%H_2O(containing 0.1%TFA), retention times of the DMAA diastereomers were 29.01 min and 29.90 min; D. 40%ACN + 60%H_2O(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_2O (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-Fl. As shown in Figure 6, dansyl-1,

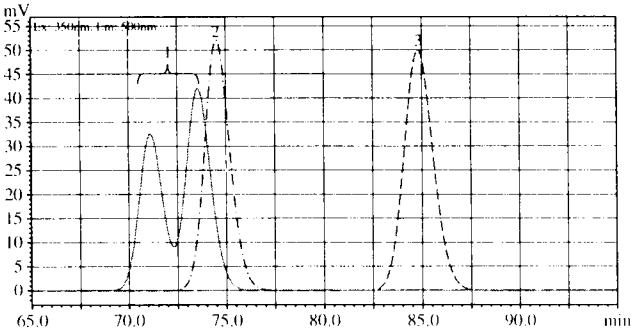


Figure 6. The fluorescence chromatograms of dansyl derivatives of DMAA and its isomers. 1. Diastereomers of dansyl-1,3-dimethylamylamine (dansyl-DMAA), 2. dansyl-1,4-dimethylpentylamine, 3. dansyl-2-aminoheptane. Mobile phase: 40%ACN + 60%H_2O(containing 0.1%TFA). Flow rate: 1 ml/min.

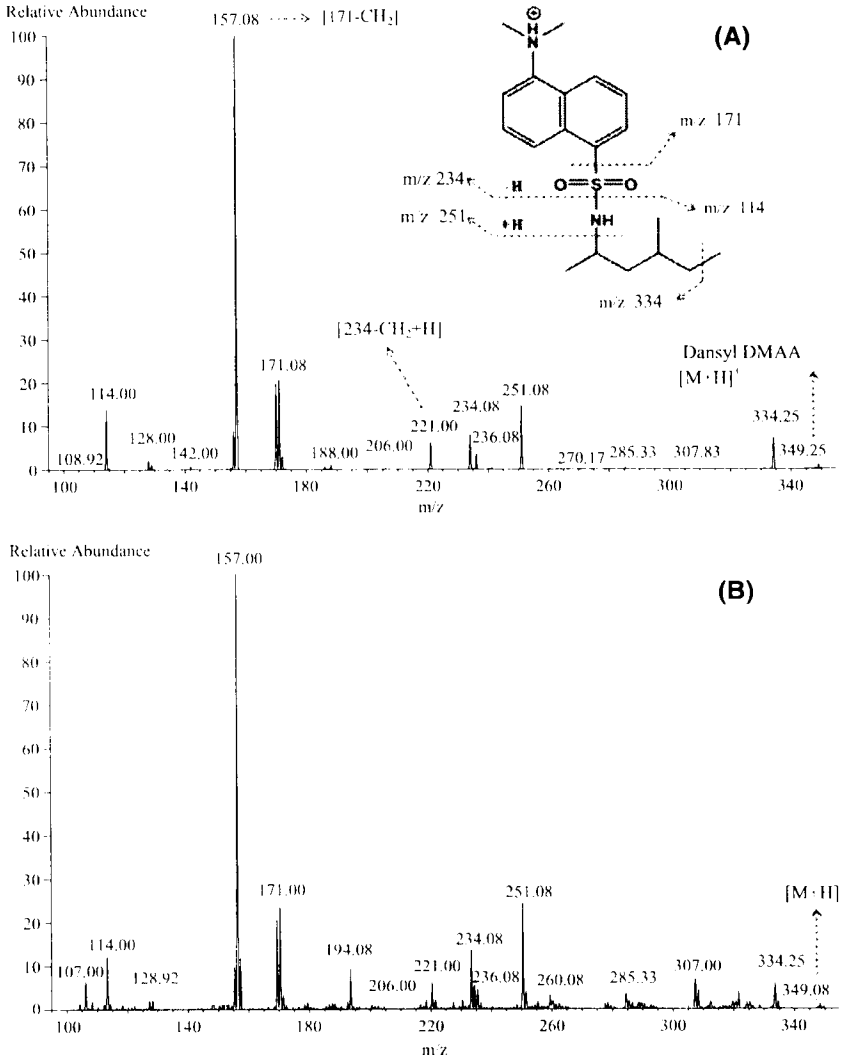


Figure 5. The tandem MS spectra of (A) dansyl DMAA standard (from ChromaDex) and (B) dansylated Now Foods geranium oil. Parent ion *m/z*: 349, width: 3.

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 Now Foods geranium oil and the Earth Solutions geranium oil, respectively.

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

[1] H.A. Shonle, E. Rohrmann. US Patent No 2350318, **1944**.
[2] E. Rohrmann, H.A. Shonle. Amino alkanes as pressor substances. *J. Am. Chem. Soc.* **1944**, *66*, 1516.
[3] Anonymous. New and nonofficial remedies: methylhexamine; forthane. *J. Am. Med. Assoc.* **1950**, *143*, 1156.
[4] Anonymous. What to do about DMAA? *Nutr. Bus. J.* **2012**, *17*, 1.
[5] P. Zang, J. Qing, Q. Lu. A study on the chemical constituents of geranium oil. *Guizhou Gongxueyuan Xuebao* **1996**, *25*, 82.
[6] World Anti-doping Agency. WADA 2010 Prohibited List. Available at: http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf [19 September 2009].
[7] NDTV. Now nine Aussie athletes test positive for methylhexaneamine. Available at: http://www.ndtv.com/article/commonwealth%20games/now-nine-aussie-athletes-test-positive-for-methylhexaneamine-61768&cp [23 October 2010].
[8] P. Cossins. Rui Costa and his brother test positive. Available at: http://www.cyclingnews.com/news/rui-costa-and-his-brother-test-positive [19 October 2010].
[9] BBC Sport. Second Nigerian tests positive at Commonwealth Games. Available at: http://news.bbc.co.uk/sport2/hi/commonwealth_games/delhi_2010/9082481.stm [12 October 2010].
[10] P. Gee, S. Jackson, J. Easton. Another bitter pill: a case of toxicity from DMAA party pills. *N. Z. Med. J.* **2010**, *123*, 124.
[11] T.J. Tritten. Army probing connection between body building supplement, 2 deaths. Available at: http://www.stripes.com/news/army-probing-connection-between-body-building-supplement-2-deaths-1.163652 [15 December 2011].
[12] P. Chiaramonte. Soldier deaths during training prompt military probe into supplement use. Available at: http://www.foxnews.com/us/2012/02/02/soldier-deaths-during-training-sparks-military-probe-into-supplement-use/ [2 February 2012].
[13] E. Watson. UNPA: We agree with AHPA on DMAA labeling. Available at: http://www.nutraingredients-usa.com/Industry/UNPA-We-agree-with-AHPA-on-DMAA-labeling [10 January 2012].
[14] D.F. Marsh, A. Howard, D.A. Herring. The comparative pharmacology of the isomeric nitrogen-methyl-substituted heptylamines. *J. Pharmacol. Exp. Ther.* **1951**, *103*, 325.
[15] R.B. Stoughton, G. Deoreo, W. Clendenning. Effects of intradermal injection of vasopressors in normal and diseased human skin. *Arch. Dermatol.* **1960**, *82*, 400.
[16] D.T. Walz, T. Koppanyi, G.D. Maengwyn-Davies, M.L. Joyce. Isoproterenol vasomotor reversal by sympathomimetic amines. *J. Pharmacol. Exp. Ther.* **1960**, *129*, 200.
[17] NCI. In vivo screening data. Available at: http://dtp.nci.nih.gov/dtpstandard/servlet/InvivoScreen?testshortname=Tumor+LE+(ip)+in+06&searchlist=1106&searchtype=NSC [18 March 2012].
[18] S. Daniells. Health Canada: DMAA is not from geranium. Available at: http://www.nutraingredients-usa.com/Industry/Health-Canada-DMAA-is-not-from-geranium [24 August 2011].
[19] E. Adelson. Real Heat Wave Risk Posed by Fake "Geranium". Available at: http://www.thepostgame.com/features/201108/geranium-products-might-pose-serious-risk-athletes [15 August 2011].
[20] S. Starling. Food? Medicine? Neither? UK agencies "trying to get to bottom" of DMAA status. Available at: http://www.nutraingredients.com/Regulation/Food-Medicine-Neither-UK-agencies-trying-to-get-to-bottom-of-DMAA-status/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=Newsletter%2BDaily&c=gaa%2FSjqPjaAEIOqUhpyCi%2BX4aUxc3sFA [23 February 2012].
[21] Anonymous. Geranium oil research. **2012**. Available at: http://dmaare-search.com/geranium-oil-research [18 March 2012].
[22] A. Lisi, N. Hasick, R. Kazlauskas, C. Goebel. Studies of methylhexaneamine in supplements and geranium oil. *Drug Test. Anal.* **2011**, *3*, 873.
[23] E. Watson. USPLabs: DMAA is from geranium oil - and critics are "uninformed". Available at: http://www.nutraingredients-usa.com/Regulation/USPLabs-DMAA-is-from-geranium-oil-and-critics-are-uninformed [5 January 2012].
[24] D.W. Armstrong, X. Wang, J. Lee, Y. Liu. Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco. *Chirality* **1999**, *11*, 82.
[25] T. Mroczek, K. Glowniak, A. Wlaszczyk. Simultaneous determination of N-oxides and free bases of pyrrolizidine alkaloids by cation-exchange solid-phase extraction and ion-pair high-performance liquid chromatography. *J. Chromatogr. A* **2002**, *949*, 249.
[26] L. Zhou, A.A. Hopkins, D.V. Huhman, L.W. Sumner. Efficient and sensitive method for quantitative analysis of alkaloids in hardinggrass (Phalaris aquatica L.). *J. Agric. Food Chem.* **2006**, *54*, 9287.

Exhibit 18

The highlighted portions of the attached exhibit illustrate the portions of the Zhang/Armstrong article that were altered from the unpublished version.

Research article

Drug Testing and Analysis

Received: 20 March 2012 Revised: 6 April 2012 Accepted: 9 April 2012 Published online in Wiley Online Library

www.drugtestinganalysis.com DOI 10.1002/dta.1368

1,3-Dimethylamylamine (DMAA) in supplements and geranium products: natural or synthetic?

Ying Zhang, Ross M. Woods, Zachary S. Breitbach 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 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*).

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.[1–3] 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.[4] 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.[5]

In 2009, DMAA was added to the 2010 prohibited list by the World Anti-Doping Agency (WADA) since it is a stimulant.[6] 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.[7–9] 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.[10] 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.[11,12] 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.[4] This statement was supported by the United Natural Products Alliance (UNPA) in January 2012.[13]

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[14] and 1960[15,16] as well as the National Cancer Institute (NCI) *in vivo* screening data of synthetic DMAA[17] 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.[18,19] Other debated questions are whether DMAA should be regulated or even be allowed in supplements.[4,20] 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[5]: (1) It mislabelled DMAA as 2-hexanamide, 4-methyl-.[4,21] (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 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

* *Correspondence to: Daniel Armstrong, Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019. E-mail: sec4dwa@uta.edu*

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019, USA

Table 1. Geranium oils tested

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

arise from the addition of synthetic material.[22] Also, another researcher in National Science Foundation Internationals failed to detect DMAA from several geranium essential oils available on the market.[4] Despite this criticism, USPlabs insisted that DMAA in its products, Jack3d and OxyElite, was from geranium.[23]

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.[24] 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 trifluroacetic 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).

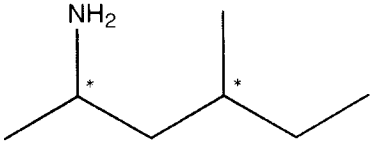


Figure 1. The structure of 1,3-dimethylamylamine (DMAA).

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

Drug Testing and Analysis

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 × 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-electospray ionization-triple quadrupole (HPLC-ESI-QQQ) analysis, the extracted residue was dansylated prior to analysis. In the dansylation procedure, the extracted residue was dissolved in 3 mL of 2:1 acetone:3M sodium carbonate. Then 25 mg 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 × 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 1mL/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 distereomeric ratios and quantification of DMAA content in supplements were operated at 90 °C isothermally. 2-Aminopentane was used as internal standard in the quantification of DMAA in the supplements. The enantiomeric 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 Thermo LXQ linear ion trap mass spectrometer and a LARIHC CF6-P column (15cm · 2.1mm) (AZYP, Arlington, TX, USA) were used. The HPLC mobile phase consisted of 90% acetonitrile containing 0.1% formic acid and 10% methanol containing 0.1% formic acid. The total flow rate for HPLC was 0.4 mL/min. The mass spectra data were recorded in a positive mode of electrospray ionization for selected ion *m/z* 116.2. Capillary voltage and spray voltage were set at 25 V and 5 kV, respectively. The injection volume was 5 µL. The limit of detection (LOD) of this method was 50 parts per billion (ppb) of spiked DMAA in geranium oils.

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)

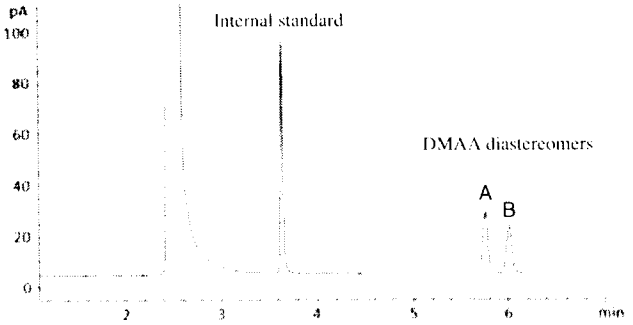


Figure 2. GC chromatogram of N-pentafluoropropionyl derivatives of DMAA standard (from Sigma-Aldrich) and internal standard at 90 C. Diastereomeric ratio = Peak Area A/ Peak Area B. The retention times of the internal standard, DMAA diastereomer A and B were 3.62 min, 5.78 min, and 6.20 min, respectively.

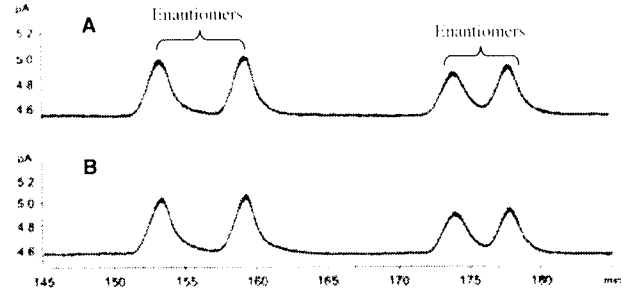


Figure 3. GC chromatograms of N-pentafluoropropionyl DMAA enantiomers at 30 C. A)Synthetic DMAA, B) DMAA in the supplement manufactured by Primaforce. DMAA in all the other supplements had identical chromatograms as B).The retention times for the four enantiomers were 153.49 min, 159.18 min, 174.11 min and 177.71 min, respectively.

(Phenomenex, Torrance, CA, USA) were used. The HPLC mobile phase consisted of 70% of acetonitrile and 30% of H_2O containing 0.1% trifluoroacetic acid. The total flow rate was 0.2 mL/min. The mass spectra data were recorded in the positive ion mode (multiple reaction monitoring transition of *m/z* 349 to 171 corresponding to the primary dansyl fragment and neutral loss of sulfonyl-DMAA). The collision energy was −25 volts with argon as the collision gas. The injection volume was 5 µL. The LOD of this method was 10 ppb.

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 were indistinguishable.

The concentrations of DMAA in most of the supplements were fairly high. In general, the concentrations of molecules with low

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

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

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

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. 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 signal of DMAA was greatly suppressed by the other geranium oil components remaining in the residue. However, the LOD was significantly reduced when the components in the samples were separated via HPLC before entering the mass spectrometer.

For the underivatized samples, a LARIHC CF6-P column was used for the separation. This column provided adequate retention of primary amines when using polar organic mobile phases.[27,28] The retention time of DMAA in this column was about 8 min. (See Experimental and Supporting Information.) The LOD of this HPLC-ESI-LIT 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 oils 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

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

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

[1] H.A. Shonle, E. Rohrmann. US Patent No 2350318, **1944**.
[2] E. Rohrmann, H.A. Shonle. Amino alkanes as pressor substances. *J. Am. Chem. Soc.* **1944**, *66*, 1516.
[3] Anonymous. New and nonofficial remedies: methylhexamine; forthane. *J. Am. Med. Assoc.* **1950**, *143*, 1156.

[4] Anonymous. What to do about DMAA? *Nutr. Bus. J.* **2012**, *17*, 1.
[5] P. Zang, J. Qing, Q. Lu. A study on the chemical constituents of geranium oil. *Guizhou Gongxueyuan Xuebao* **1996**, *25*, 82.
[6] World Anti-doping Agency. WADA 2010 Prohibited List. Available at: http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf [19 September 2009].
[7] NDTV. Now nine Aussie athletes test positive for methylhexaneamine. Available at: http://www.ndtv.com/article/commonwealth%20games/now-nine-aussie-athletes-test-positive-for-methylhexaneamine-61768&cp [23 October 2010].
[8] P. Cossins. Rui Costa and his brother test positive. Available at: http://www.cyclingnews.com/news/rui-costa-and-his-brother-test-positive [19 October 2010].
[9] BBC Sport. Second Nigerian tests positive at Commonwealth Games. Available at: http://news.bbc.co.uk/sport2/hi/commonwealth_games/delhi_2010/9082481.stm [12 October 2010].
[10] P. Gee, S. Jackson, J. Easton. Another bitter pill: a case of toxicity from DMAA party pills. *N. Z. Med. J.* **2010**, *123*, 124.
[11] T.J. Tritten. Army probing connection between body building supplement, 2 deaths. Available at: http://www.stripes.com/news/army-probing-connection-between-body-building-supplement-2-deaths-1.163652 [15 December 2011].
[12] P. Chiaramonte. Soldier deaths during training prompt military probe into supplement use. Available at: http://www.foxnews.com/us/2012/02/02/soldier-deaths-during-training-sparks-military-probe-into-supplement-use/ [2 February 2012].
[13] E. Watson. UNPA: We agree with AHPA on DMAA labeling. Available at: http://www.nutraingredients-usa.com/Industry/UNPA-We-agree-with-AHPA-on-DMAA-labeling [10 January 2012].
[14] D.F. Marsh, A. Howard, D.A. Herring. The comparative pharmacology of the isomeric nitrogen-methyl-substituted heptylamines. *J. Pharmacol. Exp. Ther.* **1951**, *103*, 325.
[15] R.B. Stoughton, G. Deoreo, W. Clendenning. Effects of intradermal injection of vasopressors in normal and diseased human skin. *Arch. Dermatol.* **1960**, *82*, 400.
[16] D.T. Walz, T. Koppanyi, G.D. Maengwyn-Davies, M.L. Joyce. Isoproterenol vasomotor reversal by sympathomimetic amines. *J. Pharmacol. Exp. Ther.* **1960**, *129*, 200.
[17] NCI. In vivo screening data. Available at: http://dtp.nci.nih.gov/dtpstandard/servlet/InvivoScreen?testshortname=Tumor+LE+(ip)+in+06&searchlist=1106&searchtype=NSC [18 March 2012].
[18] S. Daniells. Health Canada: DMAA is not from geranium. Available at: http://www.nutraingredients-usa.com/Industry/Health-Canada-DMAA-is-not-from-geranium [24 August 2011].
[19] E. Adelson. Real Heat Wave Risk Posed by Fake "Geranium". Available at: http://www.thepostgame.com/features/201108/geranium-products-might-pose-serious-risk-athletes [15 August 2011].
[20] S. Starling. Food? Medicine? Neither? UK agencies "trying to get to bottom" of DMAA status. Available at: http://www.nutraingredients.com/Regulation/Food-Medicine-Neither-UK-agencies-trying-to-get-to-bottom-of-DMAA-status/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=Newsletter%2BDaily&c=gaa%2FSjqPjaAEtOqUhpyCi%2BX4aUxc3sFA [23 February 2012].
[21] Anonymous. Geranium oil research. **2012**. Available at: http://dmaare-search.com/geranium-oil-research [18 March 2012].
[22] A. Lisi, N. Hasick, R. Kazlauskas, C. Goebel. Studies of methylhexaneamine in supplements and geranium oil. *Drug Test. Anal.* **2011**, *3*, 873.
[23] E. Watson. USPLabs: DMAA is from geranium oil - and critics are "uninformed". Available at: http://www.nutraingredients-usa.com/Regulation/USPLabs-DMAA-is-from-geranium-oil-and-critics-are-uninformed [5 January 2012].
[24] D.W. Armstrong, X. Wang, J. Lee, Y. Liu. Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco. *Chirality* **1999**, *11*, 82.
[25] T. Mroczek, K. Glowniak, A. Wlaszczyk. Simultaneous determination of N-oxides and free bases of pyrrolizidine alkaloids by cation-exchange solid-phase extraction and ion-pair high-performance liquid chromatography. *J. Chromatogr. A* **2002**, *949*, 249.
[26] L. Zhou, A.A. Hopkins, D.V. Huhman, L.W. Sumner. Efficient and sensitive method for quantitative analysis of alkaloids in hardinggrass (Phalaris aquatica L.). *J. Agric. Food Chem.* **2006**, *54*, 9287.
[27] P. Sun, D.W. Armstrong. Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan6 chiral stationary phase. *J. Chromatogr. A* **2010**, *1217*, 4904.
[28] P. Sun, C. Wang, Z.S. Breitbach, Y. Zhang, D.W. Armstrong. Development of new HPLC chiral stationary phases based on native and derivatized cyclofructans. *Anal. Chem.* **2009**, *81*, 10215.

Exhibit 19

News & Events

FDA NEWS RELEASE

For Immediate Release: April 27, 2012
Media Inquiries: Tamara Ward, 301-796-7567, tamara.ward@fda.hhs.gov
Trade Press Inquiries: Sebastian Cianci, 240-402-2291, sebastian.cianci@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

FDA challenges marketing of DMAA products for lack of safety evidence

Agency cites ten companies in warning letters

The U.S. Food and Drug Administration today issued warning letters to ten manufacturers and distributors of dietary supplements containing dimethylamylamine, more popularly known as DMAA, for marketing products for which evidence of the safety of the product had not been submitted to FDA.

Also referred to as 1,3-dimethylamylamine, methylhexanamine, or geranium extract, the ingredient is in dietary supplements and is often touted as a "natural" stimulant.

The companies receiving warning letters and their product names are:

Company	Product(s)
Exclusive Supplements[1]	Biorhythm SSIN Juice
Fahrenheit Nutrition[2]	Lean Efx
Gaspari Nutrition[3]	Spirodex
iSatori Global Technologies, LLC[4]	PWR
Muscle Warfare, Inc.[5]	Napalm
MuscleMeds Performance Technologies[6]	Code Red
Nutrex Research[7]	Hemo Rage Black Lipo-6 Black Ultra Concentrate Lipo-6 Black Lipo-6 Black Hers Ultra Concentrate Lipo-6 Black Hers
SEI Pharmaceuticals[8]	MethylHex 4,2
SNI LLC[9]	Nitric Blast
USP Labs, LLC[10]	Oxy Elite Pro Jack3D

"Before marketing products containing DMAA, manufacturers and distributors have a responsibility under the law to provide evidence of the safety of their products. They haven't done that and that makes the products adulterated," said Daniel Fabricant, Ph.D., Director of FDA's Dietary Supplement Program.

Specifically, the warning letters cite the companies for marketing products for which a notification had not been submitted for the use of DMAA as a New Dietary Ingredient (NDI). Under current law, dietary supplement manufacturers or distributors who use certain dietary ingredients not marketed in a dietary supplement prior to October 15, 1994, are responsible for notifying the FDA of evidence to support their conclusion that their dietary supplements containing NDIs are safe. Manufacturers or distributors must submit notification at least 75 days before marketing their products. The companies warned today were marketing products for which this requirement had not been met.

The FDA warning letters also advised the companies that the agency is not aware of evidence or history of use to indicate that DMAA is safe. Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), manufacturers, marketers and distributors of dietary supplements are responsible for ensuring that they are marketing a safe product.

The FDA letters noted that DMAA is known to narrow the blood vessels and arteries, which can elevate blood pressure and may lead to cardiovascular events ranging from shortness of breath and tightening in the chest to heart attack. The agency has received 42 adverse event reports on products containing DMAA. While the complaints do not establish that DMAA was the cause of the incidents, some of the reports have included cardiac disorders, nervous system disorders, psychiatric disorders, and death.

The agency additionally warned the companies that synthetically-produced DMAA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement. DSHEA defines a dietary ingredient as a vitamin, mineral, amino acid, herb or other botanical, a dietary substance for use by man to supplement the diet, or a concentrate, metabolite, constituent, extract, or combination of these substances.

The companies have 15 business days to respond to the FDA with the specific steps they will take to address the issues in the warning letters.

For more information:

How dietary supplements are regulated[11]

Dietary Supplement Health and Education Act of 1994[12]

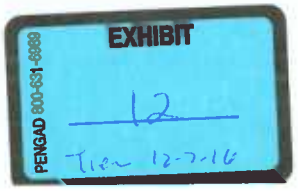
New Dietary Ingredient notification process[13]

Reporting adverse events associated with FDA regulated products[14]

#

The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

Read our Blog: FDA Voice[15]



Visit the FDA on Facebook[16] [17], Flickr[18] [19], YouTube[20] [21] and Twitter[22] [23]

RSS Feed for FDA News Releases[24]

Page Last Updated: 05/01/2012
Note: If you need help accessing information in different file formats, see Instructions for Downloading Viewers and Players.

U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Ph. 1-888-INFO-FDA (1-888-463-6332)
Email FDA

Links on this page:

1. /ICECI/EnforcementActions/WarningLetters/2012/ucm302162.htm
2. /ICECI/EnforcementActions/WarningLetters/2012/ucm302261.htm
3. /ICECI/EnforcementActions/WarningLetters/2012/ucm302211.htm
4. /ICECI/EnforcementActions/WarningLetters/2012/ucm302202.htm
5. /ICECI/EnforcementActions/WarningLetters/2012/ucm302160.htm
6. /ICECI/EnforcementActions/WarningLetters/2012/ucm302279.htm
7. /ICECI/EnforcementActions/WarningLetters/2012/ucm302165.htm
8. /ICECI/EnforcementActions/WarningLetters/2012/ucm302295.htm
9. /ICECI/EnforcementActions/WarningLetters/2012/ucm302078.htm
10. /ICECI/EnforcementActions/WarningLetters/2012/ucm302167.htm
11. /Food/DietarySupplements/default.htm
12. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148003.htm
13. /Food/DietarySupplements/NewDietaryIngredientsNotificationProcess/default.htm
14. /Safety/ReportaProblem/ConsumerComplaintCoordinators/default.htm
15. https://blogs.fda.gov/fdavoice/
16. http://www.facebook.com/FDA
17. http://www.fda.gov/AboutFDA/AboutThisWebsite/WebsitePolicies/Disclaimers/default.htm
18. http://www.flickr.com/photos/fdaphotos/
19. http://www.fda.gov/AboutFDA/AboutThisWebsite/WebsitePolicies/Disclaimers/default.htm
20. http://www.youtube.com/user/USFoodandDrugAdmin?blend=23&ob=5
21. http://www.fda.gov/AboutFDA/AboutThisWebsite/WebsitePolicies/Disclaimers/default.htm
22. http://twitter.com/us_fda
23. http://www.fda.gov/AboutFDA/AboutThisWebsite/WebsitePolicies/Disclaimers/default.htm
24. /AboutFDA/ContactFDA/StayInformed/RSSFeeds/PressReleases/rss.xml

Exhibit 20

Stimulant Potentially Dangerous to Health, FDA Warns

FDA's efforts to get dietary supplements containing the stimulant DMAA off the market illustrates the agency's role in regulating dietary supplements and serves as a warning to consumers.

The Food and Drug Administration (FDA) is using all available tools at its disposal to ensure that dietary supplements containing a stimulant called dimethylamylamine (DMAA) are no longer distributed and available for sale to consumers in the marketplace.

The ingredient, DMAA, is most commonly used in supplements promising weight loss, muscle building and performance enhancement; it can elevate blood pressure and could lead to cardiovascular problems, including heart attack, shortness of breath and tightening of the chest. Given the known biological activity of DMAA, the ingredient may be particularly dangerous when used with caffeine.

As of April 11, 2013, FDA had received 86 reports of illnesses and deaths associated with the supplement containing DMAA. The majority are voluntary reports from consumers and healthcare practitioners. The illnesses reported include heart problems and nervous system or psychiatric disorders. Note, however, that a report is not proof that the product actually caused the problem.

FDA has warned companies known to be using DMAA in dietary supplements that those products containing this ingredient are illegal. Such warnings offer the quickest way at FDA's

"Consumers may mistakenly look at a capsule and think that FDA has signed off on that product as safe and effective prior to that product appearing on the market ..."

disposal to halt the further distribution of dietary supplements containing DMAA in the marketplace. In fact, all but one of the companies sent a Warning Letter have agreed to stop using DMAA as an ingredient in their dietary supplements. The one company that has yet to agree to such action, USPLabs, has responded to FDA's warning by submitting published studies that purport to challenge FDA's conclusions.

However, after reviewing the studies provided by USPLabs, FDA has found the information insufficient to defend the use of DMAA as an ingredient in dietary supplements. FDA is finalizing a formal response to the firm to reflect its findings, according to Daniel Fabricant, Ph.D., director of FDA's Division of Dietary Supplement Program.

FDA's authority over dietary supplements is very different from its authority over drugs and other medical products. FDA is required to undertake what are usually lengthy scientific and legal steps in order to force the removal of dietary supplements that may be unsafe or are otherwise illegal if companies don't voluntarily comply.

As FDA continues the process needed to get DMAA off the market, the agency is urging consumers to check labels and avoid any dietary supplements containing DMAA, which is referred to on different product labels by 10 possible names. The alternatives are listed at FDA's DMAA web page (*http://www.fda.gov/Food/DietarySupplements/QADietarySupplements/ucm346576.htm*).

The Challenge

FDA's response to the use of DMAA illustrates the challenges that the agency faces in addressing incidents involving potentially dangerous dietary supplements. The effort is increasingly important as the use of dietary supplements increases worldwide. A 2011 study (*http://www.cdc.gov/nchs/data/databriefs/db61.htm*) found that more than half of U.S. adults used a dietary supplement between 2003 and 2006, compared to 40% between 1988 and 1994.

In recent years, FDA has alerted consumers to hundreds of tainted products marketed as dietary supplements. Consumers should be aware that dietary supplements are subject to different oversight than drugs and other medical products.

"Consumers may mistakenly look at a capsule and think that FDA has signed off on that product as safe and effective prior to that product appearing on the market, as we do with drugs and other medical products," says Fabricant. "In contrast, with dietary supplements, there is no premarket approval, and once a product is on the market, the burden is on the FDA to prove that a product is unsafe."

FDA's role in overseeing dietary supplements is laid out in a 1994 law and subsequent amendments. FDA's enforcement capabilities range from issuing warning letters seeking voluntary cooperation—the quickest way to get a product off the market—to bringing criminal charges. In recent years, FDA enforcement actions involving dietary supplements have included banning products, executing injunctions, working with U.S. marshals to seize products, and issuing safety alerts and consent decrees—which are agreements approved and enforced by a federal court.

In many cases, FDA has acted when dietary supplements were found to contain ingredients approved for use in prescription drugs. DMAA was approved in 1948 for use as a nasal decongestant, but the approval was withdrawn in 1983.

The products cited in the warning letter to USPLabs are Oxy Elite Pro and Jack3D. These products claim, among other things, to be fat-burning and performance-enhancing supplements, respectively. While action in that case in pending, FDA is following up to ensure that other companies which promised to cease using DMAA as an ingredient in their dietary supplements are actually doing so. FDA is also looking to see if there are other dietary supplement products containing DMAA in the marketplace, and will continue to act to ensure that such products, when identified, are no longer distributed and available for sale to consumers.

Consumers are urged to report any problems associated with supplement use to the company or the agency (*http://www.fda.gov/Safety/MedWatch/default.htm*), and to always consult with their health care professional before using a supplement. FDA

Find this and other Consumer Updates at *www.fda.gov/ForConsumers/ConsumerUpdates*

Sign up for free e-mail subscriptions at *www.fda.gov/consumer/consumerenews.html*

Exhibit 21

EXHIBIT FILED UNDER SEAL

Exhibit 22

Analytical Chemistry Insights

RAPID COMMUNICATION

Identification and Quantification of Dimethylamylamine in Geranium by Liquid Chromatography Tandem Mass Spectrometry

J.S. Li, M. Chen and Z.C. Li

Intertek-AAC Labs, 711 Parkland Court, Champaign, Illinois, 61821.
Corresponding author email: z.charlie.li@intertek.com

Abstract: A sensitive and reliable method of liquid chromatography–electrospray ionization/tandem mass spectrometry (LC-ESI/MS/MS) was developed and validated for determining 1,3-dimethylamylamine (1,3-DMAA) and 1,4-dimethylamylamine (1,4-DMAA) in geranium plants (*Pelargonium graveolens*). The sample was extracted with 0.5 M HCl and purified by liquid-liquid partition with hexane. The parameters for reverse-phase (C18) LC and positive ESI/MS/MS were optimized. The matrix effect, specificity, linearity, precision, accuracy and reproducibility of the method were determined and evaluated. The method was linear over a range of 0.10–10.00 ng/mL examined, with R^2 of 0.99 for both 1,3-DMAA and 1,4-DMAA. The recoveries from spiked concentrations between 5.00–40.00 ng/g were 85.1%–104.9% for 1,3-DMAA, with relative standard deviation (RSD) of 2.9%–11.0%, and 82.9%–101.8% for 1,4-DMAA, with RSD of 3.2%–11.7%. The instrument detection limit was 1–2 pg for both DMAAs. The quantification limit was estimated to be 1–2 ng/g for the plant sample. This method was successfully applied to the quantitative determination of 1,3- and 1,4-DMAA in both geranium plant and geranium oil.

Keywords: 1,3-dimethylamylamine, 1,4-dimethylamylamine, geranium (*Pelargonium graveolens*), liquid chromatography-tandem mass spectrometry (LC/MS/MS)

Analytical Chemistry Insights 2012:7 47–58

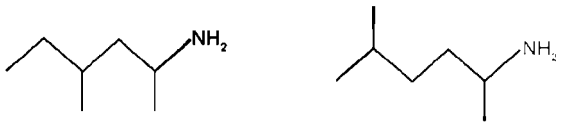
doi: 10.4137/ACI.S9969

This article is available from http://www.la-press.com.

Introduction

Dimethylamylamine (DMAA), also known as methylhexaneamine, or 1,3-dimethylpentylamine, is a simple aliphatic amine (Fig. 1). In recent years, it has appeared as an ingredient in various dietary supplements and sports nutrition products. This use of the compound has given rise to inquiries as to whether DMAA is a naturally occurring constituent of the geranium (*Pelargonium graveolens*) plant and its oil. Consequently, an investigation was undertaken to make an identification and determination of DMAA in the plant and its oil, if present.

The result of a GC/MS analysis of geranium oil, which indicated the presence of 1,3- DMAA (1) and 1,4-DMAA (2) was published in a journal that has not been widely circulated internationally.[1] Aside from that publication, geranium essential oil has been the subject of numerous other investigations seeking to identify and quantify all of the compounds present, generally employing the use of GC/FID and GC/MS; these other investigations have failed to identify 1,3-DMAA (1) or 1,4-DMAA (2).[2–12] However, because geranium oil presents such a complex sample matrix and contains many volatile compounds, a possible shortcoming of the GC/FID method is interference by the sample matrix.[6] Although the use of GC/MS is an improvement, the complex nature of the sample matrix still presents issues when seeking to identify all components of the oil;[6] in fact, we are not aware of any published data demonstrating an identification of components comprising 100% of the oil. These factors, along with differences in sample origin, processing and composition, may explain why these other investigations failed to identify DMAA as a component of geranium oil. Regarding 1,3-DMAA (1), it has been noted that although it is amenable to GC/MS analysis,[13] great care must be taken during method development because of DMAA's strong polarity, volatility and low molecular mass, making it a challenge for GC-column retention and separation.



1,3-dimethylamylamine (1) 1,4-dimethylamylamine (2)

Figure 1. Structures of 1,3-Dimethylamylamine (1) and 1,4-Dimethylamylamine (2).

DMAA has been successfully determined in urine using LC/MS/MS.[13,14] In those investigations, 1,3-DMAA (1) was detected as a pair of peaks with identical MS/MS spectra. However, the determination of DMAA in plant and especially in geranium oil has yet to be published. In the present study, we have developed and validated a sensitive and simple LC/MS/MS method to identify 1,3-DMAA (1) and 1,4-DMAA (2) and determine their concentrations as naturally occurring compounds in geranium plants.

Experimental

Chemicals and reagents

1,3-Dimethylamylamine (1) (CAS No. 105-41-9, >99%) and 1,4-dimethylamylamine (2) (CAS No. 28292-43-5, >99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (88%), methanol (HPLC grade), hexane (pesticide residue grade) and hydrochloric acid (37% HCl) were all obtained from Fisher Scientific (Waltham, MA, USA). All other chemicals are analytical grades. Water (18.2 MΩ-cm) was prepared with a Barnstead NanoPure Diamond System (Lake Balboa, CA, USA).

Apparatus and instruments

Agilent 1100 HPLC system with quaternary pump (Santa Clara, CA, USA) was coupled to a Micromass Quattro Ultima mass spectrometer with electrospray ionization (ESI) source (Manchester, England). Masslynx (version 4.1) were used to control the system of LC-triple quadrupole mass spectrometer and for data acquisition and processing. The analytical column is a Phenomenex Kinetex C18 column (4.6 × 150 mm, 2.6 µm) (Torrance, CA, USA). High Speed Grinder DFY-200 was from Gaoyi In. (Wenzhou, China). Commercial Blender 200G was from Waring Co. (Torrington, CT, USA).

Geranium plants and geranium oils

The geranium plants were procured by and obtained from Dr. Yi Jin of Yunnan University (Kunming, Yunnan Province, China) and were authenticated by Professor Xu Youkai of the Xishuangbanna Tropical Botanical Garden-Chinese Academy of Sciences (Mengla, Yunnan Province, China). The plant samples were collected from different areas of China

(*see* Table 5), shipped to this lab fresh and stored at –20 °C immediately upon receipt in the lab. All geranium oil samples were obtained from Jiangxi Ji'an Hengcheng Flavor Oil Factory (Ji'an, Jiangxi Province, China). The geranium oil was stored at room temperature.

Standard preparation

The stock solutions of 1,3-DMAA (1) and 1,4-DMAA (2) were prepared separately in methanol at a concentration of 1.00 mg/mL and 1.068 mg/mL, respectively. Working solutions composed of the two DMAAs were prepared by serial dilution of the stock solution with 0.5 M HCl to obtain a set of standard concentrations of 0.10–10.00 ng/mL of 1,3- DMAA (1), and 0.11–10.68 ng/mL of 1,4-DMAA (2). All working standard solutions were stored at 4 °C and used within one week after preparation, although no significant degradation was observed in one month of storage.

Sample preparation and extraction

Geranium plant (wet leaves and stems) was thawed at room temperature and cut into pieces at about 1–2 cm and mixed well. After about five hundred grams of the plant was ground into fine pieces with a high speed grinder, 10 g of sample was weighed into a stainless steel blender cup. To this cup, 80 mL of 0.5 M HCl was added and mixed. The mixture was homogenized at high speed for 2 min. The homogenate was then transferred into a 100-mL volumetric flask. The blade and cup were washed with additional 15 mL of 0.5 M HCl. The solution was collected into the flask and extracted by sonication at 50 °C for 1 hour. After being cooled to room temperature, the volume was adjusted to the mark with 0.5 M HCl. This solution was centrifuged at 4000 × g for 10 min, and the supernatant was further purified as below.

For geranium oil, 1 mL of sample was mixed with 1 mL of hexane in a 10-mL glass tube with screw cap. Five mL of 0.5 M HCl was added and shaken with a vortex shaker for 5 min at high speed. The aqueous layer (lower) was diluted with 0.5 M HCl as necessary, filtered with a 0.45-µm nylon filter and applied to LC/MS/MS without further purification.

Purification

Four mL of supernatant and 2 mL of hexane were added to a 10-mL glass tube with screw cap. The mixture was shaken by a vortex shaker for 30 sec. The mixture was centrifuged at 2000 × g for 5 min. The aqueous layer was diluted as necessary and filtered for LC/MS/MS analysis.

LC/MS/MS conditions

The mobile phase of HPLC is composed of Water:acetonitrile (85:15) containing 0.1% formic acid. Flow rate was 0.5 mL/min; column temperature was 35 °C; the flow was diverted 0.2 mL/min to MS. Injection volume was 50 µL.

The mass spectrometer was operated in positive ESI and multiple reaction monitoring (MRM) mode. Nitrogen was used as the nebulizer, heater, and cone gas. Argon was used as the collision induced dissociation (CID) gas. The precursor-to-product ion transitions were monitored at m/z 116 [M + H] → 57 (quantification) and m/z 116→99 (qualification) for both 1,3-DMAA (1) and 1,4-DMAA (2). ESI parameters were optimized for maximizing the generation and stability of the precursor and fragment ions by infusion as follows: Capillary 2.5 kV, Cone 20V, Source temperature 120 °C, Desolvation temperature 360 °C, Cone gas 120 L/hour, Desolvation gas 850 L/hour, CID 11 eV, collision cell pressure 2 × e-3 mbar.

Method validation

The analytical method was validated according to guidelines for United States Pharmacopeia (USP). The parameters validated include linearity, specificity, limit of detection, limit of quantification, accuracy, precision and reproducibility.

Linearity

To evaluate the linearity, calibration curves of 1,3-DMAA (1) and 1,4-DMAA (2) were established using concentrations in the range from 0.1 to 10 ng/mL. The responses of each compound against its respective concentration were plotted. Linear regression analysis was performed to obtain calibration equation and correlation coefficients (R^2).

Specificity

Two ion transitions coupled with a high resolution column were used to enhance the method selectivity. Specificity was assessed by comparing the chromatograms of blanks (glassware and reagent blanks), standard, spiked samples and their peak purity (peak

shape and relative intensity of transitions). The peak was identified by retention time and relative intensity of transitions against the reference standard.

Matrix effects

The matrix effects (ion suppression or enhancement) were evaluated by comparing peak area of the standard, sample extract and the extract directly spiked at corresponding concentrations of DMAAs, which were set at the medium spiked concentration (20 ng/g).

Accuracy

Accuracy of the method was determined by assaying spiked samples of geranium plants at four different levels: about 5, 10, 20 and 40 ng/g for both 1,3-DMAA (1) and 1,4-DMAA (2) (ie, adding 50, 100, 200 or 400 ng DMAA in solution to about 10 g of sample). Each concentration level had three replicates. All samples were extracted, purified and determined as described above. For the blank, 10 mL of water instead of sample was included and carried through the same procedures of sample preparation.

Precision and reproducibility

Precision was performed by assaying a geranium sample in six subsamples. The concentration of each subsample, and average and RSD of the analyses were calculated to access the precision of the method.

Reproducibility of this method was evaluated by a second chemist beside the primary chemist in this laboratory. The geranium sample was assayed in six subsamples. The concentrations, average and RSD of the six analyses were compared with the results obtained by the primary chemist to assess the reproducibility of the method by a different chemist.

Data analysis

The concentrations of 1,3-DMAA (1) and 1,4-DMAA (2) in the sample preparations were obtained from their corresponding standard curves. The mean, standard deviation (SD) and relative standard deviation (RSD) of spike recoveries were calculated for assessing the accuracy of the method. Mean and RSD of repeated analyses of the geranium samples were used for precision evaluation. The mean concentration of DMAAs from the precision experiments was used as the original value to calculate recoveries of DMAAs. The recoveries from spiked samples were calculated by the following formula:

$$\text{Recovery, \%} = 100\ (\text{Fc} - \text{Bc})/\text{Sc}$$

where, Fc is the concentration (ng/g) found in the spiked sample; Bc is the original value of the sample (ng/g) prior to spiking; Sc (ng/g) is the concentration spiked to the sample.

Results and Discussion

Analytical conditions

Because DMAA is a simple aliphatic amine with no chromatic group, the HPLC-UV method is not suitable for its detection unless the compound is derivatized with a chromophore prior to analysis. Therefore, LC/MS/MS was selected for the current method. Two solvent systems, methanol-water and acetonitrile-water were tested for optimization on the C18 column and tuning of ESI/MS. Both solvent systems produce similar ESI (+) signals. Further investigations showed that formic acid (0.1%) in either mobile phase enhances the signal significantly, with little effect on retention time and shape of the peaks. As a result, acetonitrile:water (15:85) containing 0.1% formic acid was selected and used routinely as mobile phase in the current experiment. Under these conditions, 1,3-DMAA (1) and 1,4- DMAA (2) were well separated with good peak shape (tailing factor = 1.1–1.3) and retention ($K' = 2.5–2.8$) (Fig. 2).

When a standard solution of 1,3-DMAA (1) was analyzed under the current conditions, double peaks with similar intensity were observed. Similar results were observed by Vorce et al[13] and Perenoud et al.[14] Both peaks show identical CID mass spectra (data not shown), suggesting that these peaks are stereo isomers (Fig. 2A). In contrast, 1,4-DMAA (2) shows only a single peak (Fig. 2B). 1,3-DMAA (1) has two chiral centers (carbon-1 and carbon-3) in its structure, and thus it theoretically has four stereo isomers. The double peaks observed are likely formed by its diastereomers, (1*S*,3*S*)/(1*R*,3*R*) and (1*S*,3*R*)/(1*R*,3*S*). These diastereomers have different molecular shapes or stereo-hindrance effects when they interact with the stationary phase. For example, they may be resolved on a reverse-phase column.[15] In contrast, 1,4-DMAA (2) has only one chiral center at carbon-1.

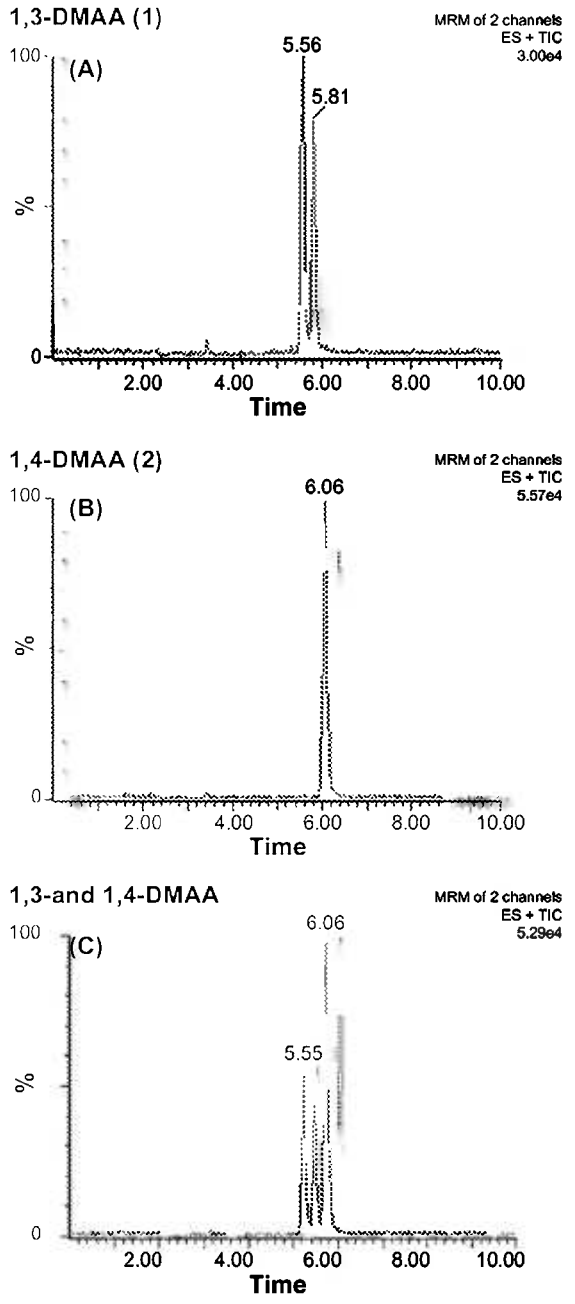


Figure 2. MRM chromatograms of (**A**) 2 ng/mL 1,3-DMAA (1) (the peaks of a pair of diastereomers at 5.5 min and 5.8, respectively); (**B**) 2.67 ng/mL 1,4-DMAA (2) (6.1 min); (**C**) the mixture.

Its enantiomers [(1*S*,1*R*) and (1*R*,1*S*)] have the same chemical and physical properties, and therefore show a single peak under the current analytical conditions. Perrenoud et al[14] further confirmed that the double peaks were diastereomers by their identical chemical shifts of ^{13}C NMR and ^{1}H NMR.

Figure 3 shows that 1,3-DMAA (1) and 1,4-DMAA (2) produced similar CID mass spectra for the same precursor ion m/z 116 $[M+H]^+$). Their CID spectra had a strong and stable product ion m/z 57 $[C_4H_9]^+$ that was used for quantification. Other product ions with relatively higher abundance were m/z 43 $[C_3H_7]^+$, m/z 75 and m/z 99 $[M+H-NH3]^+$.

Sample preparation, matrix effect and specificity

DMAA is slightly soluble in water, but soluble in diluted HCl and many polar solvents. Both 0.5 M HCl and methanol were examined for their extraction of the geranium samples. It was found that both solvents had similar extraction efficiency. However, methanol extracts much more of the matrix components from the geranium plant than 0.5 N HCl. The final extract prepared using methanol was found to interfere with LC/MS analysis (data not shown). In contrast, the extract prepared using 0.5 N HCl carries less matrix components, especially fat-soluble compounds, which was later found to be important for the accuracy of determination. Therefore, the

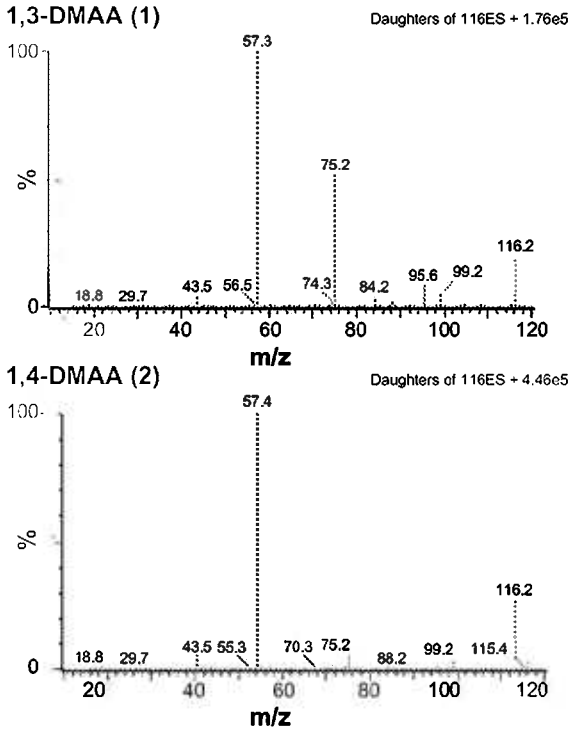


Figure 3. The CID spectra (11 eV) of 1,3-DMAA (1) (Top); 1,4-DMAA (2) (Bottom).

diluted HCl solution was used for routine sample extraction. Furthermore, sonication for 60 minutes of the geranium sample was found to be necessary for reproducible results.

The recovery of DMAAs in initial experiments was found to be low (about 60%–70%) when the HCl-extract was directly applied to LC/MS/MS without further purification. To investigate the cause of this low recovery, our focus was on the effect of the sample matrix, since the ESI signal is more susceptible to matrix-induced suppression. Results of the investigation clearly showed that the matrix of the sample had suppressed the signal of DMAAs in the sample extract or spiked sample extract (Fig. 4). For example, after subtracting the background, the ESI signal of 1,3-DMAA (1) in the spiked extract with purification was about 35% greater than that without purification (Fig. 4B and C). These results suggested that ion suppression had occurred in the raw extract. It is also noted that ion-suppression is slightly less at the elution window for 1,4-DMAA (2), which was only 20% lower in the spiked extract without purification.

It has been known that ion suppression occurs in many ESI/MS-based methods for biological samples.[16] Methanol is a strong protic solvent and can solubilize considerable amounts of lipid components, such as fatty acids and phospholipids. Considering the ESI mechanism, these nonvolatile or less volatile compounds were the potential solutes suppressing the ESI signal.[17] The diluted HCl solution used in the current method provides strong solubility for DMAAs but solubilizes considerably less of these fat-soluble components. Furthermore, the HCl-extract is readily purified by partitioning with hexane to remove the lipid-soluble components. This simple purification step was found to be effective in reducing sample matrix and ion suppression, and improved recovery significantly (Fig. 4B and C).

No interfering peaks were observed from glassware/reagent blank samples. The 1,3-DMAA (1) (its diastereomers) and 1,4-DMAA (2) are well-resolved (Fig. 5) in the purified extracts. The DMAAs between the standards and the samples have identical retention time and similar peak relative intensity (See Table 6). These results provide evidence that the method is specific for analysis of DMAAs since

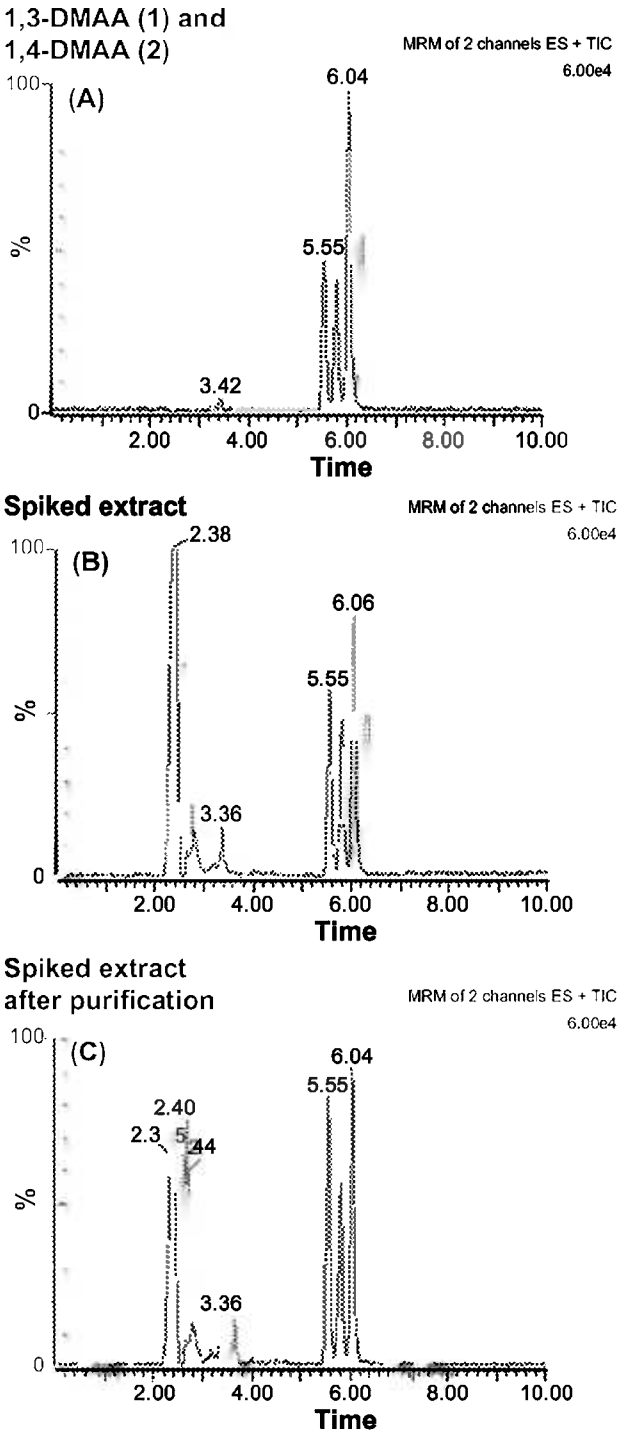


Figure 4. Matrix effect on determination of DMAAs in geranium (*Pelargonium graveolens*) plant: (**A**) 2.50 ng/mL 1,3-DMAAs (1) and 2.67 ng/mL 1,4-DMAA (2) in 0.5 HCl; (**B**) 2.5 ng/mL 1,3-DMAA (1) and 2.67 ng/mL 1,4-DMAA (2) -spiked extract before purification; (**C**) the spiked extract after purification.
Note: The ESI signals in hexane-purified extracts are significantly higher than that of extracts without purification.

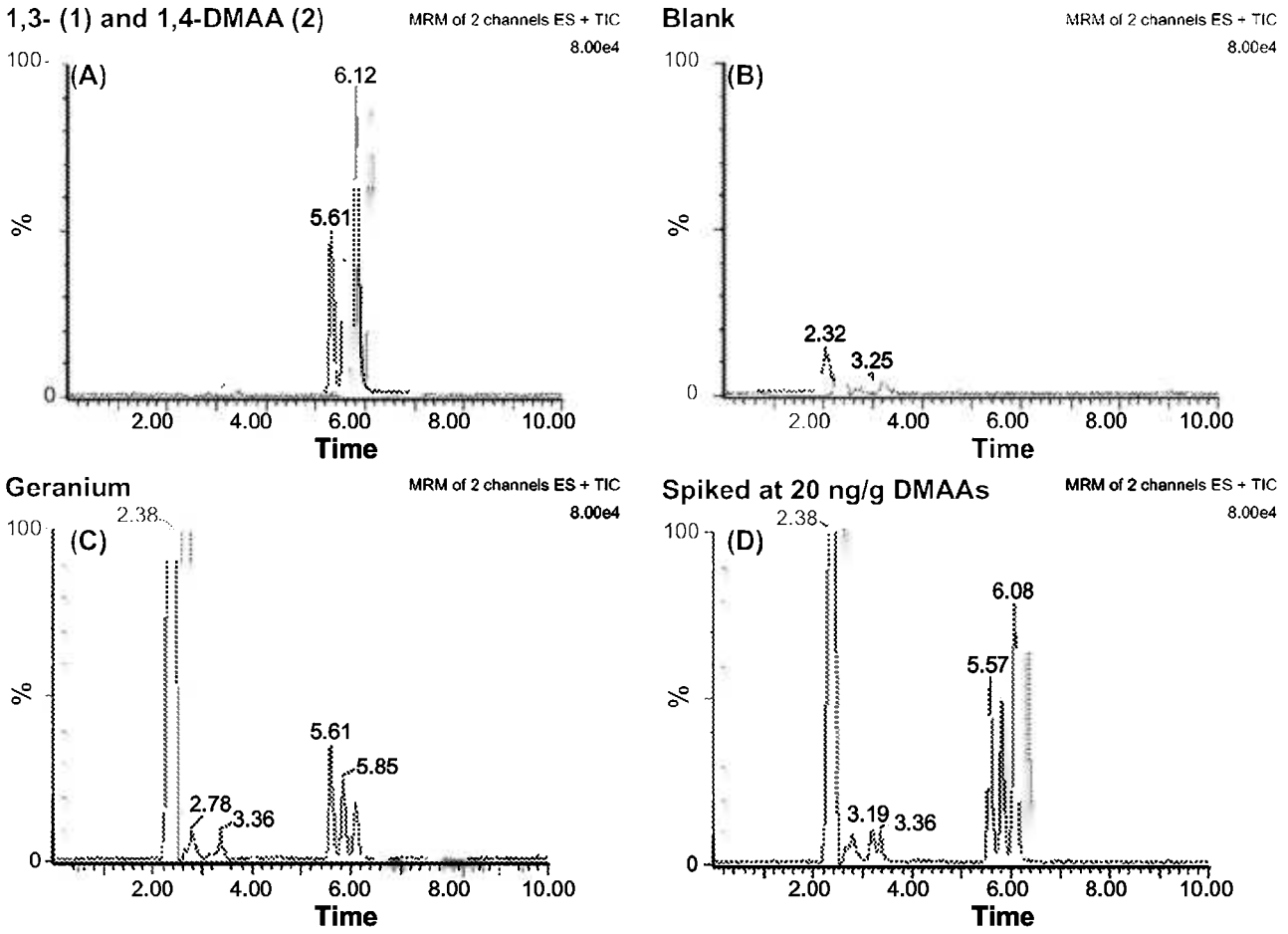


Figure 5. MRM chromatogram of (A) 2.50 ng/mL 1,3-DMAA (1) and 2.67 ng/mL 1,4-DMAA (2), (B) blank, (C) geranium (*Pelargonium graveolens*) sample and (D) 20.00 ng/g 1,3-DMAA (1) and 21.26 ng/g-spiked sample.

the matrix interference in the purified extract was not observed.

Linearity

Standard solutions of 1,3-DMAA (1) and 1,4-DMAA (2) were analyzed with concentration range at about 0.1 to 10 ng/mL. The two isomer peaks of 1,3-DMAA (1) was summed prior to regression analysis. The results were linear with R^2 of 0.998 for 1,3-DMAA (1) (Y = 2537X + 497.6, Y-peak area, X-ng/mL), and 0.999 for 1,4-DMAA (2) DMAA (Y = 2705X + 311.3).

Precision

The geranium sample showing the presence of DMAAs in initial investigation was selected for precision analysis. All the test procedures were carried out according to the method described. The results are shown in Table 1. The average amounts of 1,3-DMAA (1) and 1,4-DMAA (2) in the sample were 13.6 and 3.56 ng/g, respectively, in this geranium sample on a wet weight basis. The RSD of 1,3-DMAA (1) and 1,4-DMAA (2) are 8.3% and 5.4%, respectively. A typical MRM chromatogram (scan) of the sample is shown in Figure 5C.

Accuracy

The sample used in the precision experiment was spiked with known amounts of 1,3-DMAA (1) and 1,4-DMAA (2) for evaluating the accuracy of the method. The amounts of 1,3-DMAA (1) and 1,4-DMAA (2) (Tables 2 and 3) were used as original

Table 1. Precision for determination of 1,3-DMAA (1) and 1,4-DMAA (2) in geranium (*Pelargonium graveolens*).*

Sample no.	Weight (g)	Volume (mL)	1,3-DMAA		1,4-DMAA	
			Read (ng/mL)	Found (ng/g)	Read (ng/mL)	Found (ng/g)
1	10.59	100	1.51	14.24	0.40	3.76
2	10.66	100	1.52	14.26	0.37	3.47
3	10.88	100	1.59	14.63	0.41	3.77
4	10.62	100	1.31	12.33	0.35	3.31
5	10.11	100	1.22	12.03	0.37	3.63
6	10.34	100	1.48	14.28	0.35	3.40
Mean (ng/g)				13.63		3.56
S.D.				1.13		0.19
RSD (%)				8.3		5.4

Note: *Sample ID# 070611-0164.

Table 2. Accuracy for determination of 1,3-DMAA (1) in geranium (*Pelargonium graveolens*).

Spiked (ng)	Weight (g)	Spiked (ng/g)	Volume (mL)	Read (ng/mL)	Found (ng/g)	Recovery (%)*	Mean ± S.D.	RSD (%)
400	11.65	34.33	100	5.71	48.99	103.0	100.8 ± 2.90	2.88
	10.41	38.42	100	5.32	51.10	97.5		
	10.68	37.45	100	5.53	51.79	101.9		
200	10.11	19.78	100	3.07	30.40	84.8	86.5 ± 1.78	2.05
	10.61	18.85	100	3.18	29.94	86.5		
	10.01	19.98	100	3.13	31.28	88.3		
100	11.83	8.45	100	2.61	22.09	100.1	104.9 ± 7.52	7.16
	10.83	9.23	100	2.61	24.12	113.6		
	11.26	8.88	100	2.55	22.61	101.1		
50	10.02	4.99	100	1.77	17.71	81.8	85.1 ± 9.38	11.02
	11.05	4.52	100	1.98	17.96	95.7		
	10.06	4.97	100	1.76	17.50	77.9		

Note: *Recovery (%) = (Found-13.63)/Spiked × 100 (13.63 ng/g is the background value, See Table 1).

Table 3. Accuracy for determination of 1,4-DMAA (2) in geranium (*Pelargonium graveolens*).

Spiked (ng)	Weight (g)	Spiked (ng/g)	Volume (mL)	Read (ng/mL)	Found (ng/g)	Recovery (%)*	Mean ± S.D.	RSD (%)
427	11.65	36.67	100	5.02	43.06	107.7	101.8 ± 5.16	5.07
	10.41	41.04	100	4.62	44.42	99.6		
	10.68	40.00	100	4.57	42.82	98.1		
214	10.11	21.13	100	2.18	20.71	81.2	82.9 ± 2.66	3.21
	10.61	20.13	100	2.09	19.96	81.5		
	10.01	21.34	100	2.12	21.90	85.9		
107	11.83	9.03	100	1.42	12.04	93.9	88.6 ± 5.02	5.67
	10.83	9.86	100	1.33	12.24	88.0		
	11.26	9.48	100	1.30	11.52	83.9		
53	10.02	5.33	100	0.92	9.20	105.9	95.8 ± 10.29	10.7
	11.05	4.83	100	0.85	7.68	85.3		
	10.06	5.31	100	0.87	8.67	96.3		

Note: *Recovery (%) = (Found-3.56)/Spiked × 100 (3.56 ng/g is the background value, See Table 1).

values and taken into consideration in calculation of the recoveries (*See* "4.8.6 Data analysis"). The results are shown in Table 2 and Table 3. The average recoveries of each spiking level are 85.1%–104.9% for 1,3-DMAA (1) and 82.9%–101.8% for 1,4-DMAA (2). The RSD are 2.9%–11.0% for 1,3-DMAA and 3.2%–11.7% for 1,4-DMAA. A typical MRM chromatogram (scan) of spiked sample is shown in Figure 5D.

Detection limit and quantification limit

The instrument detection limit was estimated by analyzing a standard at a concentration of 0.2 ng/mL with injection volume of 50 µL. The chromatogram is shown in Figure 6. The detection limit was estimated to be 1–2 pg, based on the signal-to-noise ratio of 3:1.

To evaluate the method quantification limit (MQL), the signal to noise ratio of 5:1 is used for calculation. Taking into consideration of the sample weight of 10 g and the final volume of sample preparation in 100 mL, the MQL of 1,3-DMAA (1) and 1,4-DMAA (2) is estimated to be 1–2 ng/g.

Reproducibility

When the method was performed by a second chemist, similar results were obtained (Table 4). The RSD for 1,3-DMAA (1) and 1,4-DMAA (2) are 2.5% and 4.1%, respectively. These results indicate that the method is reproducible between analysts.

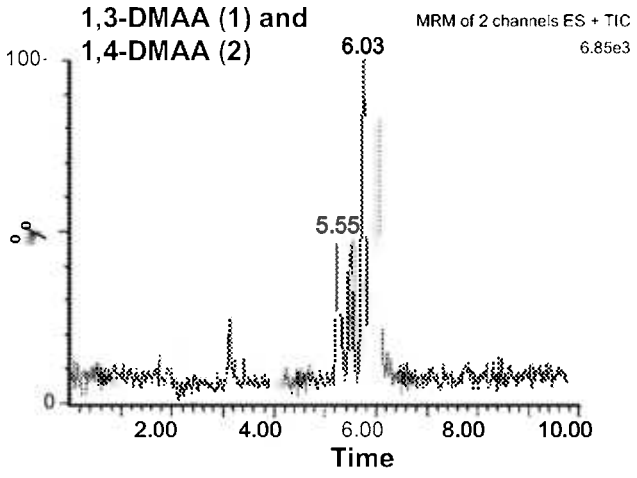


Figure 6. MRM chromatogram for estimating the instrument detection limit: 0.20 ng/mL 1,3-DMAA (1) (5.5 min and 5.8 min) and 0.11 ng/mL 1,4-DMAA (2) (6.0 min).

Table 4. Reproducibility of the procedure for 1,3-DMAA (1) and 1,4-DMAA (2) in geranium (*Pelargonium graveolens*).

Compounds	Sample (g)	Fund (ng/g)	Mean ± SD	RSD (%)
1,3-DMAA	10.51	14.24	14.00 ± 0.35	2.5
	10.80	13.81		
	10.91	13.52		
	10.76	13.84		
	10.81	14.06		
	10.78	14.51		
1,4-DMAA	10.51	4.94	4.74 ± 0.19	4.1
	10.80	5.02		
	10.91	4.56		
	10.76	4.62		
	10.81	4.58		
	10.78	4.75		

Application of the method to investigating geranium plants and geranium oils

The current method was applied to analyze geranium plants and geranium oils from different sources. The results are shown in Table 5. As expected, 1,3-DMAA (1) was further confirmed by multi-ion transition and the product ion ratios (Table 6). These results provide strong evidence that 1,3-DMAA (1) and 1,4-DMAA (2) are naturally present in both geranium plant and geranium oil.

A major advantage of the currently described method enabling detection of DMAAs in geranium plants and oils is its extreme sensitivity. The instrumental detection limit is approximate 1 pg. Another advantage is its simplicity. The method involves simple sample extraction and sample partition with hexane. There are no extended purification and derivatization steps involved, which should be necessary to GC/MS. The accuracy and precision of the method at ppb levels is easily achievable with a conventional LC column and mobile phase.

While the presence of DMAA has been reported in geranium in one investigation, those data were not considered conclusive due to issues regarding the experimental design and data analysis.[1] Therefore, to our knowledge, the present study is the first to show

Table 5. Levels of 1,3-DMAA (1) and 1,4-DMAA (2) in geranium (*Pelargonium graveolens*) plant and geranium oil from different sources.

Sample ID	Source	Date of collection	1,3-DMAA, ng/g	1,4-DMAA, ng/g
070611-0164 (plant)	Yunnan, China	June 9, 2011	13.60	3.56
072811-1026 (plant)	Jiangsu, China	June 9, 2011	165.0	35.30
072811-1027 (plant)	Guizhou, China	June 5, 2011	365.0	9.12
051911-0588 (oil)	Jiangxi, China	–	13271	220.0
042911-0988 (oil)	Jiangxi, China	–	167.0	Not detected
042911-0989 (oil)	Jiangxi, China	–	377.0	Not detected

conclusively that DMAA is naturally occurring in geranium plants.

It is well-documented that the variations of environmental conditions and geographical locations have great effect on the chemical profiles of the geranium plant.[5,8] The results in the current study showing various amounts of DMAA in geranium plants from different regions appear to be consistent with these observations (Table 5). Although the proportion of 1,3-DMAA (1) to 1,4-DMAA (2) varied considerably from sample to sample, in general, the concentration of 1,3-DMAA is much higher than that of 1,4-DMAA (2), suggesting that 1,3-DMAA (1) is the predominant form naturally occurring in geranium plants. The fact that 1,3-DMAA (1) is highly concentrated in one geranium oil sample when compared to the other two geranium oils could have been a result of either different geranium plants used for oil processing or from different manufacturing processes.

Another intriguing observation is that 1,4-DMAA (2) was not detected (below quantification limits) in two geranium oil samples where 1,3-DMAA (1) was present although at relatively lower concentrations. This discrepancy was unexpected. One explanation might be that 1,4-DMAA (2) is not stable at low concentrations. However, based on the structure of 1,3-DMAA (1) and 1,4-DMAA (2), they appear to be stable molecules with relaxed structure and no labile parts under various storage temperature conditions. Thus, an alternative explanation is that these two samples contained a higher ratio of 1,3-DMAA (1) to 1,4-DMAA (2). We have noted in the other samples, varying 1,3-DMAA (1):1,4-DMAA (2) ratios of approximately 5:1, 40:1 and 60:1, thus it is possible that with a combination of an even higher ratio and a smaller amount of 1,3-DMAA (1) present, the 1,4-DMAA (2) would have been below our quantification limits.

The results from the present study show that 1,3-DMAA (1) has two isomer peaks which are present in equal amounts and which are identical in all tested samples, including the standard reference. The reference standard of 1,3-DMAA (1) is synthetic and produced via chemical reaction. However, most compounds present in plants should be made through an enzymatic process. Therefore, most likely only one chiral configuration would be present in plants (often referred to as natural form). The results in the current study show that 1,3-DMAA (1) in geranium plants and geranium oils appears to be an exception to this notion. Indeed, this is not the first report demonstrating the presence of a racemate in a plant tissue.[18–20] In fact, the presence of a racemate (ie, nerol oxide) has been demonstrated once before in

Table 6. The relative intensity (%) of transitions for qualifying DMAAs in geranium (*Pelargonium graveolens*).

Detection ion (m/z) (precursor → product ion)		116 → 43	116 → 57	116 → 99
1,3-DMAA (1)	10 ng/mL standard	2.7	100	5.7
	072811-1026 (plant)	2.7	100	6.9
	051911-588 (oil)	3.0	100	3.7
1,4-DMAA (2)	10.68 ng/mL standard	4.1	100	4.5
	072811-1026 (plant)	3.7	100	6.1
	051911-588 (oil)	–	–	–

the geranium plant as well.[19] Further study is needed to elucidate the biosynthetic pathway of DMAAs in the geranium plant.

Conclusion

DMAA, which is used in some nutritional supplements, has led some to question whether it is actually a constituent of the geranium plant and its oils. A validated method for quantification of DMAA in geranium plants has been established in the present investigation and has confirmed the presence of 1,3-DMAA (1) and 1,4-DMAA (2) in the plant tissue and essential oil. The conditions of LC and ESI positive MS/MS have been optimized. A simple and rapid procedure for sample extraction and purification has been developed. This LC/MS/MS method is sensitive and reliable and has been used successfully for the simple and rapid analysis of DMAA in the geranium plant and its oils.

Author Contributions

J.S. Li and M Chen were responsible for data collection/analysis; J.S. Li was primarily responsible for manuscript preparation. Z.C. Li was responsible for study design and revision of manuscripts. All authors have read and approved the final manuscript.

Funding

This research was financially supported by USPlabs LLC.

Competing Interests

JSL, MC and ZCL disclose that funding for analytical research and manuscript preparation was provided by USPlabs, LLC. ZCL served as an expert witness in 2011 for the case: DeRosier v. USPlabs. Assistance with English grammar in preparation of the manuscript was provided as a courtesy to the authors by The Brewer Law Group, PLLC.

The sponsor initiated a request to this laboratory to investigate the presence of DMAA in geranium plant and geranium oil. All experimental design, method of extraction and method of quantification were carried out independently by Intertek-AAC Labs. Data analysis and the manuscript preparation were performed by the authors of the manuscript, while the sponsor provided grammatical review and assistance. The submission of the paper for publication was suggested by Intertek-AAC Labs to the study sponsor and the sponsor agreed.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

References

1. Ping Z, Jun Q. A study on the chemical constituents of geranium Oil. *J Guizhou Inst Technol*. 1996;25:82–5.
2. Babu KGD, Kaul VK. Variation in essential oil composition of rose-scented geranium (*Pelargonium* sp.) distilled by different distillation techniques. *Flavour Fragr J*. 2005;20:222–31.
3. Fayed SA. Antioxidant and Anticancer Activities of *Citrus reticulate* (*Petitgrain mandarin*) and *Pelargonium graveolens* (Geranium) Essential Oils. *Res J Agric Bio Sci*. 2009;5:740–7.
4. Gomes PB, Mata VG, Rodrigues AE. Characterization of Portuguese-grown geranium oil (*Pelargonium sp.*). *J Essent Oil Res*. 2004;16:490–5.
5. Jain N, Aggarwal KK, Syamasundar KV, Srivastava SK, Kumar S. Essential oil composition of geranium (*Pelargonium sp.*) from the plains of northern India. *Flavour Fragr J*. 2001;16:44–6.
6. Jalali-Heravi M, Zekavat B, Sereshti H. Characterization of essential oil components of Iranian geranium oil using gas chromatography-mass spectrometry combined with chemometric resolution techniques. *J Chromatogr A*. 2006;1114:154–63.
7. Kulkarni RN, Mallavarapu GR, Baskaran K, Ramesh S, Kumar S. Composition of the essential oils of two isomenthone-rich variants of geranium (*Pelargonium sp.*). *Flavour Fragr J*. 1998;13:389–92.
8. Lalli JYY, Viljoen AM, Baser KHC, Demirci B, Ozek T. The essential oil composition and chemotaxonomical appraisal of South African *Pelargoniums* (*Geraniaceae*). *J Essent Oil Res*. 2006;18:89–105.
9. Peterson A, Machmudah S, Roy BC, Goto M, Sasaki M, Hirose T. Extraction of essential oil from geranium (*Pelargonium graveolens*) with supercritical carbon dioxide. *J Chem Technol Biotechnol*. 2006;81:167–72.
10. Prakasa EVS, Rao RS, Rao G, Ramesh S. Seasonal variation in oil content and its composition in two chemotypes of scented geranium (*Pelargonium* sp.). *J Essent Oil Res*. 1995;7:159–63.
11. Shellie RA, Marriott PJ. Comprehensive two-dimensional gas chromatography-mass spectrometry analysis of *Pelargonium graveolens* essential oil using rapid scanning quadrupole mass spectrometry. *Analyst*. 2003;128:879–83.

12. Vermia RS, Verma RK, Yadav AK, Chauhan A. Changes in the essencial oil composition of rose-scented geranium (*Pelargonium graveolens* L' Herit. Ex. Ait) due to date of transplanting under hill conditions of Uttarakhand. *Indian J Nat Prod Resour*. 2010;1:367–70.
13. Vorce SP, Holler JM, Cawrse BM, Magluilo Jr J. Dimethylamylamine: a drug causing positive immunoassay results for amphetamines. *J Anal Toxicol*. 2011;35:183–7.
14. Perrenoud L, Saugy M, Saudan C. Detection in urine of 4-methyl-2-hexaneamine, a doping agent. *J Chromatogr B*. 2009;877:3767–70.
15. Dorsey JD, Dill KA. The molecular mechanism of retention in reversed-phase liquid chromatography. *Chem Rev*. 1989;89:331–4.
16. Mallet CR, Lu Z, Mazzeo JR. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. *Rapid Commun Mass Spectrom*. 2004;18:49–58.
17. Annesley TM. Ion Suppression in Mass Spectrometry. *Clin Chem*. 2003;49:1041–3.
18. Batista Jr JM, Lopez SN, Mota JS, et al. Resolution and absolute configuration assignment of a natural racemic chromane from *Peperomia obtusifolia* (*Piperaceae*). *Chirality*. 2009;21:799–801.
19. Jung DJ, Porzel A, Huneck S. Gigasol and other coumarins from *Angelica gigas*, *Phytochem*. 1991;30:710–2.
20. Wust M, Reindl J, Fuchs S, Beck T, Mosandl A. Structure elucidation, enantioselective analysis, and biogenesis of nerol oxide in Pelargonium species. *J Agric Food Chem*. 1999;47:3145–50.

Exhibit 23

EXHIBIT FILED UNDER SEAL

Exhibit 24

EXHIBIT FILED UNDER SEAL

Exhibit 25

Research article

Drug Testing and Analysis

Received: 1 August 2014 Revised: 29 August 2014 Accepted: 29 August 2014 Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1726

Methylhexanamine is not detectable in *Pelargonium* or *Geranium* species and their essential oils: A multi-centre investigation

Mahmoud A. ElSohly,[a,b,c,d]* Waseem Gul,[a,b,c] Candice Tolbert,[a] Kareem M. ElSohly,[a,b] Timothy P. Murphy,[a] Bharathi Avula,[c] Amar G. Chittiboyina,[c] Mei Wang,[c] Ikhlas A. Khan,[b,c,e] Min Yang,[f] Dean Guo,[f] Wei-Dong Zhang[g] and Juan Su[g]

In an earlier study, we developed two sensitive and reliable procedures for gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of methylhexaneamine (MHA) in *P. graveolens* plant materials and volatile oils. None of the analyzed plant materials or oils showed any detectable levels of MHA which was further substantiated by high resolution liquid chromatography-quantum time of flight-mass spectrometry (LC-QTOF-MS) analysis with a limit of detection of 10 ppb. However, other laboratories (two studies) reported the presence of MHA in some samples of *P. graveolens* and pelargonium oil acquired by the investigators from China. Because of the controversy of whether *Pelargonium* species or pelargonium oil contains MHA, it was recommended that splits of multiple samples be analyzed by different laboratories. In this investigation, multiple plant materials and oil samples were collected from around the world. These samples were submitted to four different sites for analysis. All sites adopted a similar extraction method. All the analysis sites used LC-MS/MS or LC-QTOF-MS and detection limit was set close to the 10 ng/mL as previously reported. A total of 18 plant samples belonging to 6 different *Pelargonium* species and 9 oils from different locations around the world were split among 4 different analytical laboratories for analysis (each lab received the same samples). None of the laboratories detected MHA in any of the samples at or around the 10 ppb detection level of the procedure used. Copyright © 2014 John Wiley & Sons, Ltd.

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords: MHA; *Pelargonium* species; *Geranium* species

Introduction

Methylhexaneamine (MHA), also known as dimethylpentylamine (DMPA) or dimethylamylamine (DMAA), is a simple aliphatic amine with α_1-adrenergic agonist activity reported to be 200 times less active as a vasopressor in dogs than *l*-epinephrine but with longer duration of action. Contrary to our finding of the absence of MHA in pelargonium[1], Li *et al* reported[2] low levels in samples acquired from China. In the USA and elsewhere, the compound is increasingly found in nutritional supplements, such as in weight loss and exercise 'stimulant' supplements.[3–5] In many cases, the product (s) listed the sources as geranium oil or some part of the geranium plant on the label, and in a few cases the label listed the main ingredient as MHA. Synthetic MHA can be purchased in bulk from several chemical suppliers, and can be purchased in small quantities in its pure form over the Internet. The inclusion of MHA in dietary supplements, and the alleged claim that its source is *Pelargonium* plant parts or pelargonium oil, was based on a single report in a local Chinese publication by Ping *et al*. of the presence of a small amount of the compound as part of the constituents of the volatile oil of *Pelargonium graveolens*.[6] This is in spite of the fact that there were several scientific issues with the publication and the fact that pelargonium oil was previously studied with no reports of the presence of MHA as one of its components.

Due to its purported stimulant effects, the Canadian Ministry of Health has clarified that under its regulatory systems, MHA is a drug.[7] Furthermore, the World Anti-Doping Agency (WADA) added MHA to the 2010 prohibited list.[8] MHA has resulted in a number of reported doping cases involving Indian, Nigerian, and US athletes, presumably due to the consumption of dietary supplements containing MHA.[9,10]

* *Correspondence to: Mahmoud A. ElSohly, ElSohly Laboratories, Inc., (ELI), 5 Industrial Park Drive, Oxford, MS 38655, USA. E-mail: elsohly@elsohly.com*

a *ElSohly Laboratories, Inc., (ELI), 5 Industrial Park Drive, Oxford, MS 38655, USA*

b *Phytochemical Services, Inc., (PSI), 5 Industrial Park Drive, Oxford, MS 38655, USA*

c *National Center for Natural Products Research, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

d *Divisions of Pharmaceutics, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

e *Divisions of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

f *Shanghai Institute of Materia Medica, Shanghai, China*

g *School of Pharmacy, Second Military Medical University, Shanghai, China*

In light of the dietary supplement regulations in the USA and the importance of establishing the source of their ingredients, and given the fact that MHA is a substance prohibited in many sports, and is a stimulant that reportedly carries significant health risks, it was crucial to determine whether MHA could indeed be detected in *P. graveolens* plant material or essential oil.[11,12]

In 2011, we initiated a study in which we acquired authenticated *Pelargonium* plant material (13 samples) and authenticated *Pelargonium* oil (2 samples), along with commercially available *Pelargonium* oils (20 samples) and dietary supplements labelled to contain *Pelargonium* as the source of MHA or just the chemical, MHA. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods were developed and validated, and the samples were analyzed for MHA.[1]

While all *Pelargonium* plant material and oils were found to be negative for MHA, high levels of MHA were found in the commercial dietary supplements, indicating that their source of MHA is of synthetic origin. Other investigations supported our findings.[13–16]

Meanwhile, two other laboratories, Li *et al.* and Fleming *et al.*, reported in 2012 their finding of MHA in *Pelargonium* plant and oils collected in China.[2,17] Both laboratories were supported by USP Lab, the manufacturer of dietary supplements with high levels of DMAA and both studies were published in an open access journal.

This report was initiated in order to address this controversy and to determine if there is any validity to the reports by Li *et al.* and Fleming *et al.*[2,17] Several plant samples belonging to 6 different species of the genus *Pelargonium* were acquired (18 samples in total) and several *Pelargonium* oils from different countries (9 samples total) were acquired by the National Center for Natural Products Research (NCNPR) for this study. The samples were split into four subsets of samples and one subset was submitted to each of four different analytical laboratories. All laboratories were instructed to extract the samples by a protocol similar to that of Li *et al.*, adjusting the ratio of the volume of the extraction solvent (medium) to the amount of plant material or oil to be the same as that in the Li *et al.* report.[2] Besides, each laboratory was to use its own analytical method to determine if MHA is present in any of the samples at 10 ppb level.

It must be mentioned here that since MHA has two asymmetric centres, synthetic MHA would exist two sets of diastereomers with each diasteromer composed of two enantiomers. Under normal chromatographic conditions diastereomers could be separable but enantiomers are only separable under chiral separation conditions. In our previous publication[1] we were able to separate the diastereomers of MHA on the GC-MS column as their heptafluorobutanoyl (HFB) derivatives, but not under high performance liquid chromatography (HPLC) conditions. Therefore, unless one uses chiral separation, under HPLC conditions, depending on the column, MHA would appear as either a single peak or two peaks. Zang *et al.*[13] put forth a convincing argument that the similarity of the diastereomeric ratio in the synthetic MHA as well as the enantiomeric composition of each set of diastereomers with those found in the dietary supplements is a strong indication that the MHA found in the dietary supplements is of synthetic origin.

Materials and methods

Samples

A total of 18 plant samples and 9 oils were acquired for this study by the National Center for Natural Products Research (NCNPR), the University of Mississippi. The samples are identified in Table 1 and were divided into four subsets with each subset submitted to each of the four participating laboratories. Figures 1-3 show plant material acquired from two different sources from Yunnan, China and two oil samples.

LC-MS/MS analysis of the split samples at ElSohly Laboratories (ELI)

Extraction procedure

The same general extraction procedure was used for preparation of plant material and oil samples as that reported by Li *et al.*[2] Geranium plant was cut into pieces and mixed well. The plant sample was grounded into fine pieces in a grinder, from which 1 g sample was weighed. To this, 8 mL of 0.5 M HCl was added and mixed. The mixture was homogenized and the homogenate was transferred into a 10-mL volumetric flask. The blade and cup were washed with 1.5 mL of 0.5 M HCl and transferred to the volumetric flask. The solution was extracted for 1 h by sonication at 50°C. The volume was then adjusted to 10 mL with 0.5 N HCl, transferred to centrifuge tubes and the solution was centrifuged for 10 min.

In a 10-mL screw-capped glass tube, 4 mL of supernatant and 2 mL of hexane were added and the tube capped. The tube was then shaken on a vortex shaker for 30 s and the mixture was then centrifuged for 5 min. The aqueous layer was filtered using a 0.45 μm nylon filter (Whatman), and the filtrate analyzed on the LC-MS/MS system.

For geranium oil samples, 100 μL of the oil was mixed with 100 μL of hexane in a 10 mL glass tube. To this solution, 500 μL of 0.5 M HCl was added and the mixture vortexed for 5 min on a vortex shaker. The aqueous layer was then filtered using a 0.45 μm nylon filter and analyzed on the LC-MS/MS system.

LC-MS/MS system

The LC-MS/MS system consisted of a Shimadzu Prominence HPLC with a dual pump, a vacuum solvent microdegasser, and a controlled-temperature autosampler and an MS/MS detector (Applied Biosystems/MSD Sciex Qtrap3200 with a turbo-ion ESI source operating in the positive-ion mode). The chromatographic conditions and analytical method were the same as previously described for the validated LC-MS/MS method reported by ElSohly *et al.*[1]

High resolution LC-QTOF-MS analysis of the split samples at the National Center for Natural Products Research, University of Mississippi

Extraction procedure

Samples were prepared in the same manner as described under the method used by ElSohly Laboratories in the previous section.

LC-QTOF-MS method

Chromatography was performed on an ACQUITY UPLC system (Waters Corp., Milford, MA, USA) with a temperature controlled autosampler (20°C). The injection volume was 10 μL. The separation was carried out on an ACQUITY UPLC BEH C18 Column (2.1 × 50 mm, 1.7 μm, Waters Corp., Milford, MA, USA). The column temperature was maintained at 40°C. The analysis was achieved

Table 1. Pelargonium plant material and oil samples used in the study and the results of their analysis for DMAA on LC/MS/MS (ELI), LC-QTOF-MS (NCNPR), HPLC iFunnel QTOF (School of Pharmacy at Second Military Medical University in Shanghai, China) and LC/MS/MS (ShanghaiInstitute of Materia Medica)

Sample Name	NCNPR #	ELI ACCESSION #	LC/MS/MS (ELI)	LC-QTOF-MS (NCNPR)	HPLC iFunnel QTOF (Second Military Medical University)	LC/MS/MS (Shanghai Institute)
Sample #1: *Pelargonium zonale* Aif, from Yunnan province, China		CT054	ND	ND	ND	ND
Sample #2: *Pelargonium graveolens* L'Her, Yunnan province, China		CT055	ND	ND	ND	ND
Sample #3: Provided by De'an Guo Yunnan province, China		CT056	ND	ND	ND	ND
No 1 (little bottle) :Ni de lan Rose Geranium oil Provided by Fangli Biotechnology limited company Kunming, Yunnan province		CT057	ND	ND	ND	ND
No 2 (little bottle) :Geranium oil		CT058	ND	ND	ND	ND
No 3 (little bottle) :Geranium oil-2012		CT059	ND	ND	ND	ND
No 4 (little bottle) :Geranium oil- no label		CT060	ND	ND	ND	ND
1286 commercial sample from South Africa	NCNPR# 13151	CT061	ND	ND	ND	ND
1653 (Gingind lovu, South Africa)	NCNPR# 13152	CT062	ND	ND	ND	ND
1758 (Ntsimbini, South Africa)	NCNPR# 13153	CT063	ND	ND	ND	ND
1759 (Nelspruit, South Africa)	NCNPR# 13154	CT064	ND	ND	ND	ND
1787 (Kristammahoek, South Africa)	NCNPR# 13155	CT065	ND	ND	ND	ND
Pelargonium zonale cv' daredevvk salmon' (stem)	NCNPR# 13039	CT066			ND	ND
Pelargonium zonale cv' daredevvk salmon' (leaf)	NCNPR# 13040	CT067	ND	ND	ND	ND
Pelargonium graveolens cv 'Bontrosai' (stem)	NCNPR# 13041	CT068	ND	ND	ND	ND
Pelargonium graveolens cv 'Bontrosai' (root)	NCNPR# 13042	CT069	ND	ND	ND	ND
Pelargonium graveolens cv 'Bontrosai' (leaf)	NCNPR# 13043	CT070	ND	ND	ND	ND
Pelargonium graveolens (leaf)	NCNPR #13044	CT071	ND	ND	ND	ND
Pelargonium graveolens (stem)	NCNPR #13045	CT072	ND	ND	ND	ND
Pelargonium tomentosum (leaf)	NCNPR# 13046	CT073	ND	ND	ND	ND
Pelargonium hortorum (leaf)	NCNPR# 13047	CT074	ND	ND	ND	ND
Pelargonium hortorum (stem/root)	NCNPR# 13048	CT075	ND	ND	ND	ND
Pelargonium hortorum (flower)	NCNPR# 13049	CT076	ND	ND	ND	ND
Pelargonium odoratissimum (whole plant)	NCNPR# 10591	CT077	ND	ND	ND	ND
Pelargonium tomentosum (stem)	NCNPR# 10605	CT078	ND	ND	ND	ND
Pelargonium hortorum (whole plant)	NCNPR# 10616	CT079	ND	ND	ND	ND
Pelargonium denticulatum (leaf)	NCNPR# 10636	CT080	ND	ND	ND	ND

ND: No detectable levels of DMAA/MHA.

with gradient elution using (A) acetonitrile and (B) water (both containing 0.05% formic acid) as the mobile phase at a flow rate of 0.25 mL min^{-1}. The gradient conditions were: 0–4 min linear from 5 to 70% A. The Waters ACQUITY™ XEVO QTOF Mass Spectrometer (Waters Corp., Manchester, UK) was connected to the ultra performance liquid chromatography (UPLC) system via an electrospray ionization (ESI) interface. The ESI source was operated in the positive ionization mode with the capillary voltage at 3.0 kV. The temperatures of the source and desolvation were set at 150° and 350°C, respectively. The cone and desolvation gas flows were 50 and 900 L/h, respectively. All data collected in Centroid mode were acquired using Masslynx™ NT 4.1 software (Waters Corp., Milford, MA, USA). Accurate mass calibration for positive electrospray ionization was achieved with sodium formate. For all samples analyzed, leucine-enkephalin was used as the lock mass (or reference compound), generating an $[M+H]^+$ ion (*m/z* 556.2771 and 278.1141) at a concentration of 2 ng/μL and flow rate of 5 μL/min to ensure accuracy during the MS analysis. The lock spray interval was set at 10 s and the data were averaged over five scans. The mass spectrometer was programmed to step between low (3 eV) and elevated (10 20 eV) collision energy on the gas cell, using a scan time of 1 s per function over 50 500 *m/z*. When data were acquired with MS^E, two interleaved scan functions were used. The first scan function acquired a wide mass range using low collision energy. This scan function collected precursor ion information in the sample. The second scan function acquired data over the same mass range; however, the collision energy was ramped from low to high. This scan function allowed for the collection of a full-scan accurate mass fragment with precursor ion information. MS^E data independent analysis provides accurate mass measurements of all detectable precursor and product ions which are achieved by post-acquisition lock mass corrections. All the measured masses are within 5 ppm of the theoretical value. This method involved the use of $[M+H]^+$ ions of the test compound (MHA) which was observed in the positive ion mode at *m/z* 116.1438 (calculated *m/z* = 116.1439). Further, the fragmentation patterns observed in the mass spectrum were useful in characterization of the test compound. MHA showed fragment ions at *m/z* 100.1105, 75.0257 and 57.0712. The limit of detection (LOD) for this method was estimated as 10 ppb.

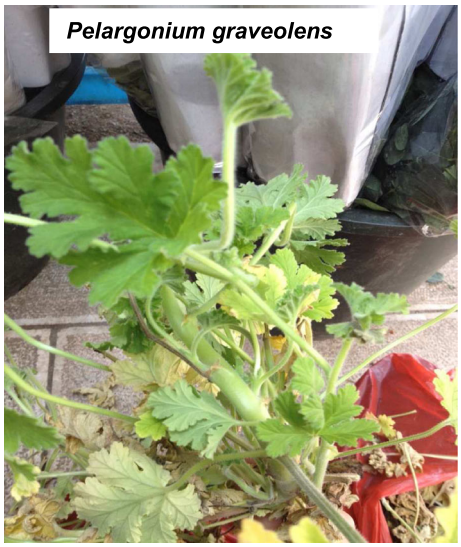


Figure 1. ***Pelargonium graveolens:*** The plant material was provided by Dr. Yi Jin at the School of Chemical Science and Technology Yunnan University, Kunming, Yunnan, China. The authentication was performed by Herbarium, Kunming Institute of Botany Chinese Academy of Science, Kunming, Yunnan, China.

Figure 2. ***Pelargonium zonale Aif:*** The plant material was purchased from Botanical garden of Haigeng State Park, Kunming, Yunnan, China and the authentication was performed by Biochemistry Department, School of Life Science, Yunnan University, Kunming, Yunnan, China.

LC-MS/MS analysis of the split samples at Shanghai Institute of Materia Medica

Extraction procedure

Samples 1 and 2 (S1 and S2): The aerial parts of fresh materials were cut to pieces and grounded with a mortar. Ten-gram samples were used for extraction. Sample 3 (S3): The aerial part of half-dried material was cut to pieces and grounded with a mortar. A 5 g sample was used for extraction. Other samples (Dried materials): The accurately weighed powdered samples were used for extraction. Oil samples: a 200-μL sample of each oil was used for extraction.

Samples were prepared in the same manner as described under the method used by ElSohly Laboratories in the previous section, with amounts of extraction solvent(s) proportioned to the weight of the samples used.

LC-MS/MS system

The analysis was performed on an Agilent 1200 HPLC coupled to an Agilent 6410 Triple-Quadrupole mass spectrometer equipped with a JetStream™ ESI source (Agilent Technologies, Inc., Santa Clara, CA, USA). Chromatographic separation was performed on a Zorbax SB 150 mm × 4.6 mm C18 column (3.5 μm particles). The column temperature was the same as room temperature. The autosampler was fitted with a 20 μL injection loop. The injection volume was 2.0 μL for control and 5.0 μL for plant material and oil. The mobile phase A was 0.1% FA in MilliQ water and mobile phase B was 0.1% FA in acetonitrile (A:B=10:90). The flow rate was 0.6 mL/min. The total run time was 15 min. The retention time of DMAA was 4.58 min. The mass spectrometer was operated in positive ESI mode. The drying gas temperature and the flow rate were 350°C and 8 L/min, respectively, and the nebulizer gas pressure was 45 psi. The capillary voltage was 4000 V. The mass spectrometer was operated in MRM mode at *m/z* 116.2 [M+H]+ → 57.1 (quantification) and *m/z* 116.2 → 41.2 (qualification) for DMAA. The fragmentor energy was 50 V and collision energy was 20 eV. Both quadrupoles mass resolution were set to 2.5 units, respectively, and the dwell times were 200 ms for each *m/z* channel. Instrument control, data acquisition and quantification were performed by MassHunter Workstation software B.03.01 (Agilent Technologies, Torrance, CA, USA).

LC/MS- QTOF analysis of the split samples at the School of Pharmacy, Second Military Medical University Shanghai China

Plant samples

Geranium plant (wet leaves and stems) was thawed at room temperature and cut into pieces at about 0.5 cm and mixed well from which 1 g was weighed into a mortar. To this mortar, 0.5 M HCl was added and mixed. The mixture was homogenized. The homogenate was then transferred into a 10-mL volumetric flask. The solution was extracted by sonication at 50°C for 1 h. After being cooled to room temperature, the volume was adjusted to the mark with 0.5 M HCl. This solution was centrifuged at 14 000r /min for 5 min, and the supernatant was further purified as below. Four mL of supernatant and 2 mL of hexane were added to a 10-mL glass tube with screw cap. The mixture was vortexed for 30 s and centrifuged at 14 000r /min for 5 min. The aqueous layer was filtered for LC-MS/MS analysis.

Oil samples

For geranium oil, 0.1 mL of sample was mixed with 0.1 mL of hexane in a 2-mL glass tube with screw cap to which 1 mL of 0.5 M HCl was added and vortexed for 5 min at high speed. The aqueous layer (lower) was filtered with a 0.45 μm nylon filter and applied to LC-MS/MS without further purification.

Preparation of standard solutions

The 1 mg/mL standard solution supplied by the NCNPR was diluted to appropriate concentrations (10, 20, 50, 100, 200, 400 ng/mL) for establishing calibration curves.

Table 2. Calibration curves LOD and LOQ of the instrument (School of Pharmacy, Second Military Medical University Shanghai China)

Analyte	Equation	Liner range (pg)	r^2	LOD (pg)	LOQ (pg)
Peak 1(MHA-1)	y=11115.80x-22907.91	100-4000	0.9998	0.8	2.8
Peak 2(MHA-2)	y=9642.87x-25111.09	100-4000	0.9999	1.0	3.0

Table 3. LOD and LOQ of the method (School of Pharmacy, Second Military Medical University, Shanghai,China)

Analyte	Plant		Oil	
	LOD (μg/kg)	LOQ (μg/kg)	LOD (ng/mL)	LOQ (ng/mL)
Peak 1(MHA-1)	28.0	93.3	5.0	16.7
Peak 2(MHA-2)	27.5	91.6	4.8	16.1

Table 4. Precision (n=2) (School of Pharmacy, Second Military Medical University, Shanghai, China)

Analyte	Plant	Oil
Peak 1(MHA-1)	5.7%	0.6%
Peak2(MHA-2)	1.2%	3.3%

Table 5. Recovery (n=2) (School of Pharmacy, Second Military Medical University, Shanghai, China)

Analyte	Sample recovery(%)		Blank recovery(%)	
	Plant	Oil	Plant	Oil
Peak 1(MHA-1)	55	94	90	98
Peak 2(MHA-2)	58	93	91	97

Figure 3. *Pelargonium graveolens* essential oil (sample on the right) was provided by Dr Yi Jin, School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan, China. *Pelargonium graveolens* essential oil; (sample on the left) was purchased from Shangi Flower Market, Kunming, Yunnan, China.

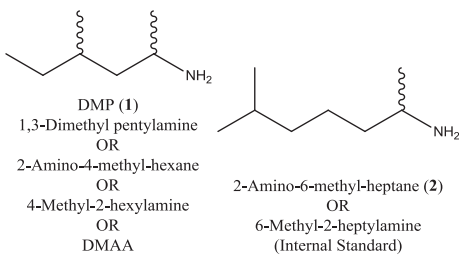


Figure 4. Chemical structures of MHA and the internal standard used for the GC-MS analysis.[1]

Preparation of recovery test samples

Plant sample: The sample (1 g) was weighed into a mortar, and then 100 μL standard solution (10 μg/mL) was added. To this, 0.5 M HCl was added and mixed. The mixture was prepared according to the plant extraction procedure as mentioned above.

Oil sample: A 10 μg/mL standard solution (100 μL) was added to 0.1 mL of oil sample in a 2-mL glass tube with screw cap, and then 0.1 mL of hexane was added. The mixture was prepared according to the oil extraction procedure as mentioned above.

Preparation of blank recovery samples

Plant sample: A 100 μL standard solution (10 μg/mL) was added into a mortar. To this mortar, 0.5 M HCl was added and mixed. The mixture was prepared according to the plant extraction procedure as mentioned above.

Oil sample: A 10 μg/mL standard solution (100 μL) was added to a 2-mL glass tube with screw cap, and then 0.1 mL of hexane was added. The mixture was prepared according to the oil extraction procedure as mentioned above.

LC-MS/MS

Agilent 1290 HPLC coupled with 6550 ifunnel QTOF was used. The analytical column used was an Agilent ZORBAX RRHD Eclipse Plus C18 column (3 mm × 100 mm, 1.8 μm) (Agilent, Santa Clara CA, USA).

The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile (0 12 min:5 8% B;12 13 min:8 95% B;13 16 min:95% B;17 19 min:5% B,*v/v*). Flow rate was 0.4 mL/min; column temperature was 30°C; Injection volume was 10 μL.

The mass spectrometer was operated in the positive ESI mode. High purity nitrogen was employed as the nebulizer, drying and sheath gas. Other parameters of the mass spectrometer were set to obtain highest intensity of protonated molecules of the analytes as follows: drying gas flow 15.0 L/min; drying gas temperature, 200°C; nebulizer pressure, 35 psi; capillary voltage, 3.5 kV; fragment voltage, 250 V; collision-induced dissociation (CID) voltage, 6 70 eV.

Validation of the procedure

The method was validated with linear range, precision, recovery, LOD, and limit of quantification (LOQ). The LOD and LOQ of the instrument were deduced by the standard solution (10 ng/mL) with

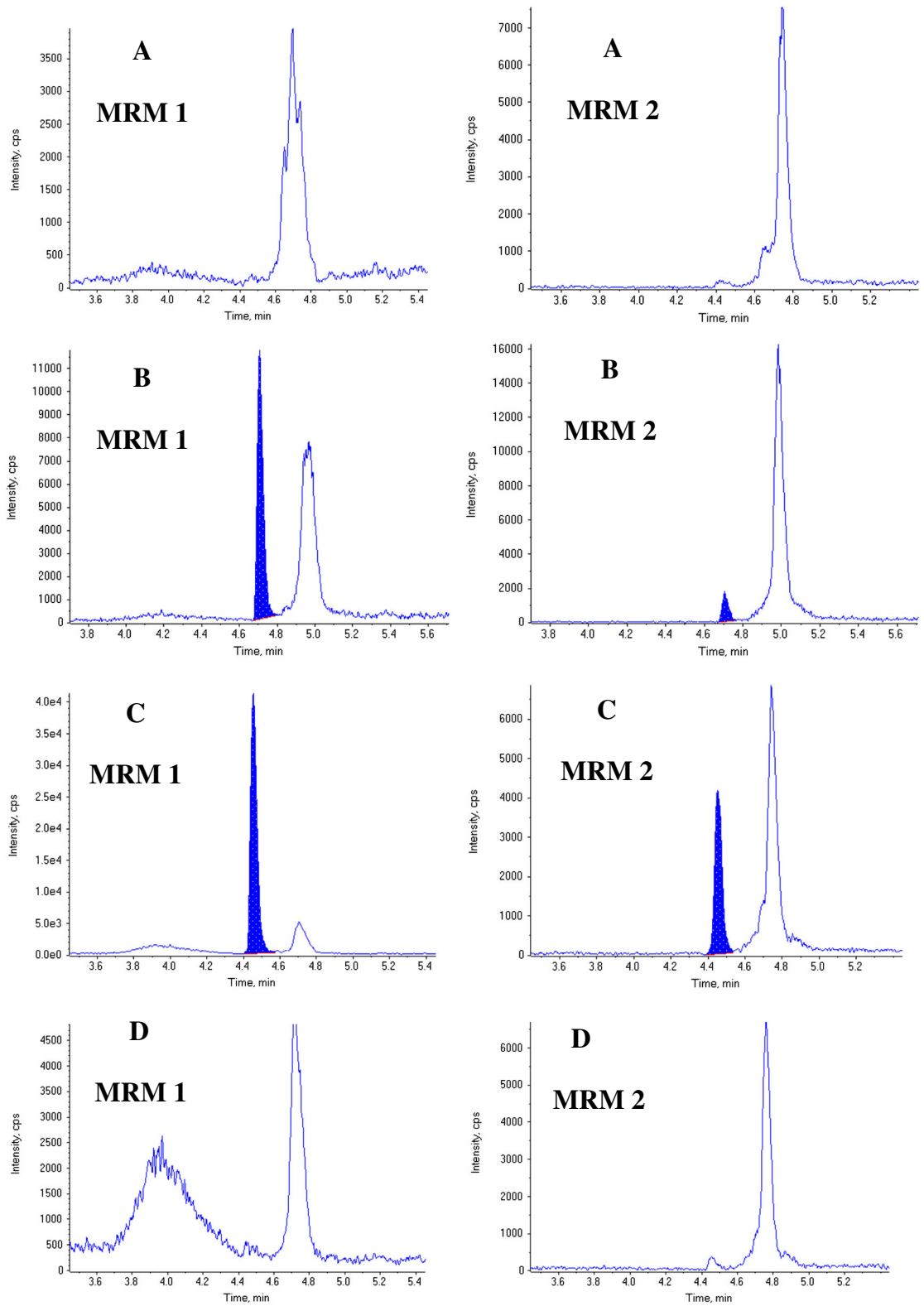


Figure 5. Chromatograms showing MRM-1 (figures on the left) and MRM-2 (figures on the right) of MHA for an unspiked sample of *Pelargonium graveolens* L'Her from Yunnan Province (**A** chromatograms), with no peaks at R_t of MHA (4.47 min) and those of the same sample spiked at 10 ng/mL MHA (showing both MRM peaks for MHA) (**B** chromatograms) and those of chromatograms **C** show a 10 ng/mL calibrator and chromatograms **D** show acetonitrile solvent blank. The highlighted peaks are those of MHA. These chromatograms are generated at ELI.

S/N 3:1 and 10:1, respectively. The LOD and LOQ of the method were deduced by recovery sample. The results are shown in Tables 2–5.

Sample analysis

Eighteen samples of plant materials and 9 samples of oil were analyzed by the above method. MHA was not detected in any of the 27 samples. The results are shown in Table 1. Representative chromatograms from the analysis of plant samples are shown in Figure 6, while examples of the analysis of oil samples are provided with the supporting documents.

Results and discussion

The controversy around the presence or absence of MHA in pelargonium plant material or oil started with a report, in a non-reviewed

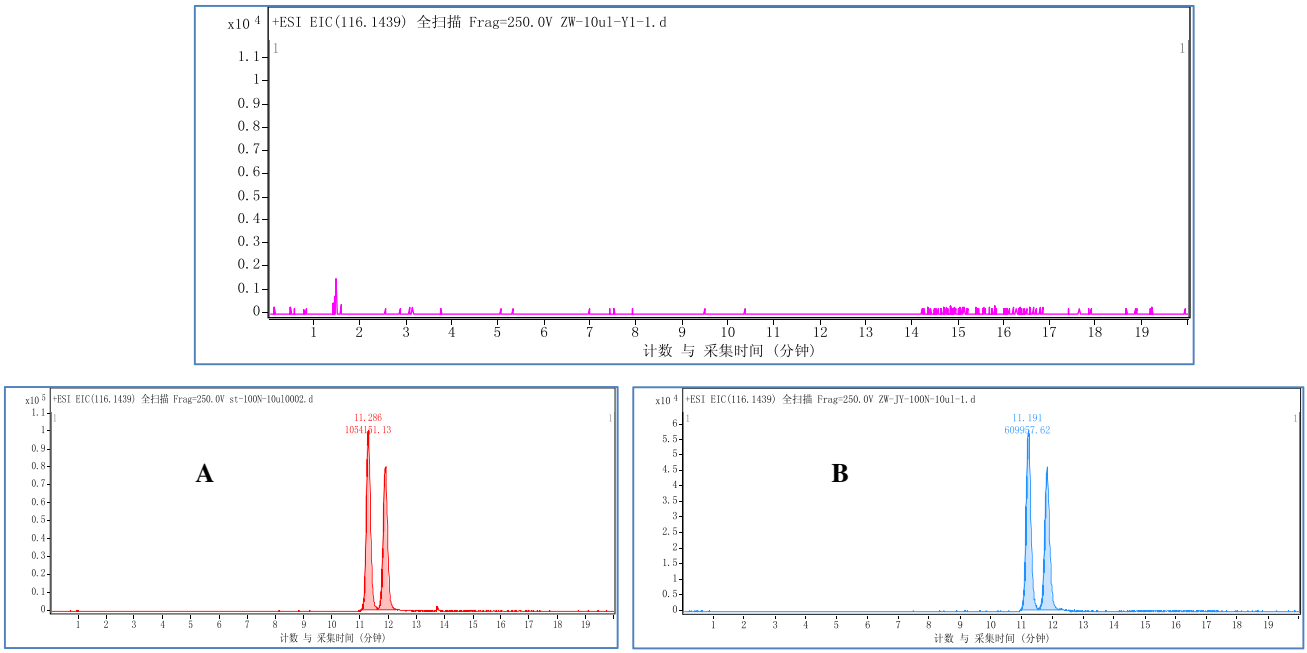


Figure 6. EIC of the extract of Plant 1 (top figure) and those of a MHA standard (bottom left, **A**) and the extract of a plant sample spiked with 1 μg of MHA (bottom right, **B**) (School of Pharmacy, Second Military Medical University Shanghai China).

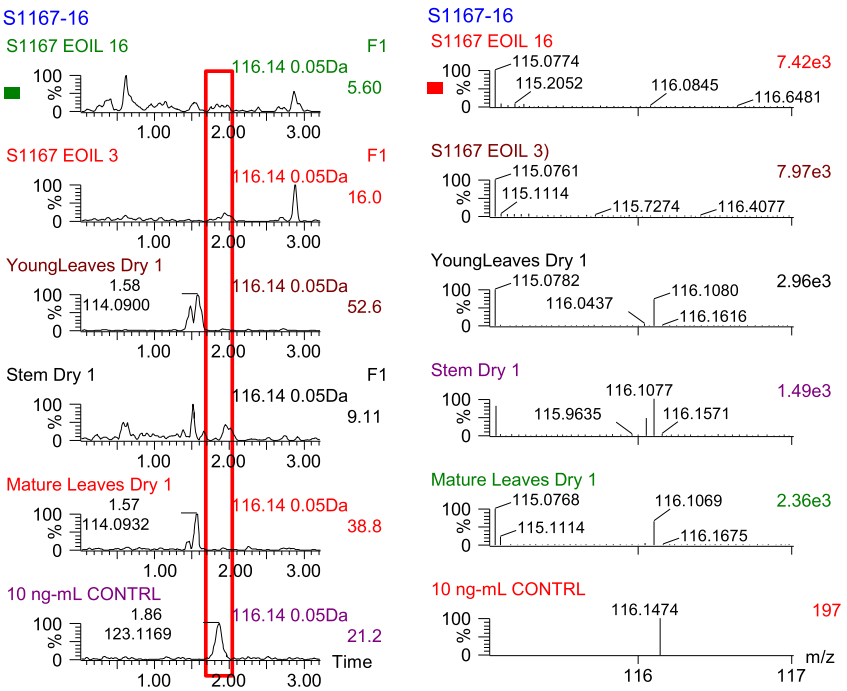


Figure 7. LC-MS-QTOF chromatograms of plant materials, oil samples and a 10 ng/mL control and the full scan accurate masses for the peaks at R_t of MHA. Note: The peak was around 1.84 min and the mass was found to be 116.10 for all samples analyzed [(where the MHA mass should be 116.14 (accurate mass = 116.1439)].

journal without proper controls, of the presence of small levels of MHA in geranium oil.[6] Because of the importance of establishing whether MHA is actually a natural product that could be sourced naturally in dietary supplements and the forensic implications associated with this issue, we conducted a study to answer this question.[1] Figure 4 shows the chemical structure of MHA and that of the I.S. used for the GC-MS analysis in our previous publication.[1] The results of our investigation (both by GC-MS and LC-MS/MS) revealed that MHA does not exist in authentic samples of *Pelargonium graveolens* plant material or pelargonium oil or in multiple samples of commercially available *Pelargonium* oil down to the ppb level. It was therefore, our conclusion that the high levels of MHA present in dietary supplements must be of synthetic origin. As such, and in the absence of a New Dietary Ingredient application with the US Food and Drug Administration (FDA), the FDA forced the dietary supplement industry to pull products containing MHA off the market.

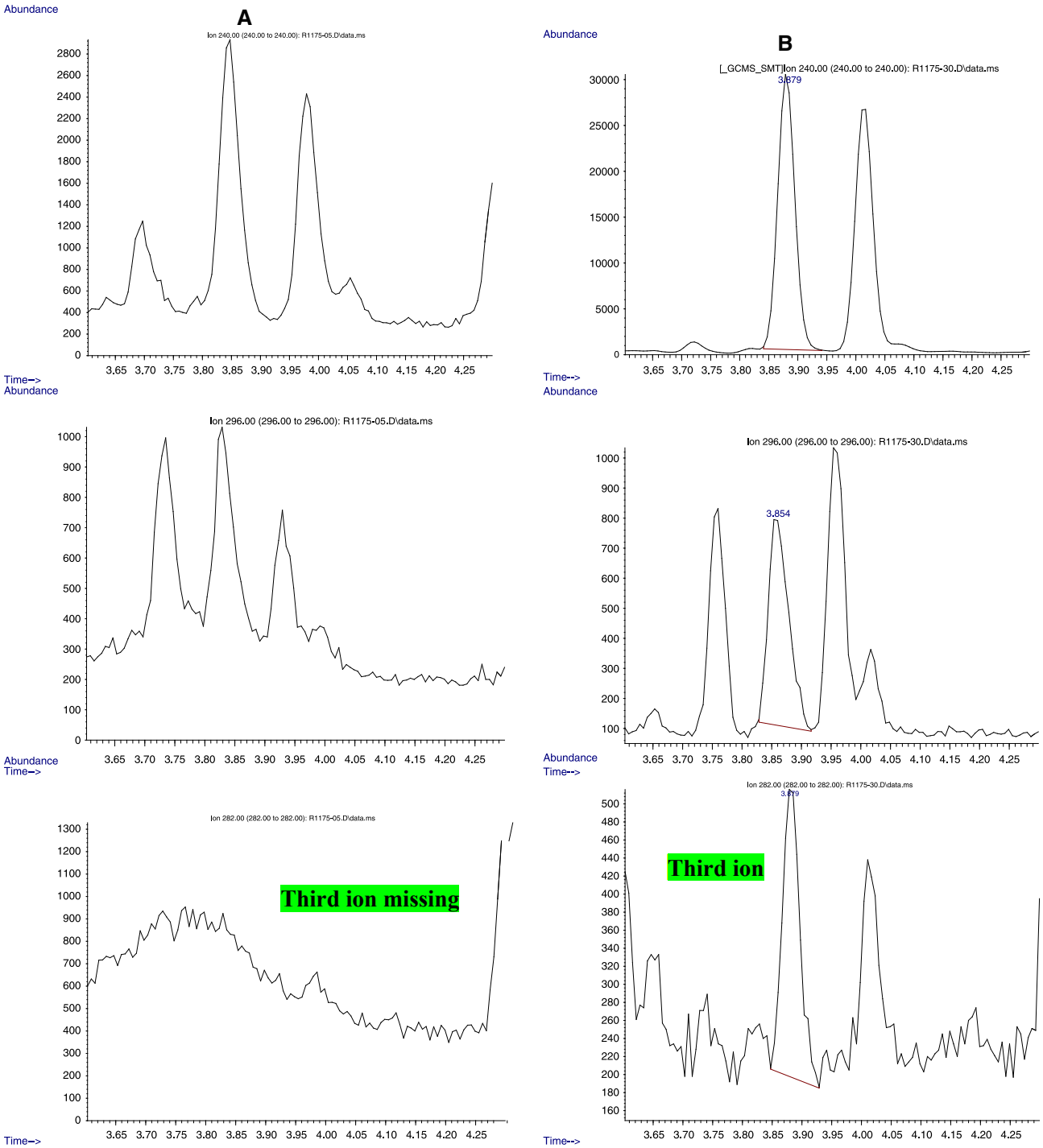


Figure 8. **(A)** GC-MS selected ion chromatograms of ions at *m/z* 240, 296, and 282 of the MHA-HFB derivative of a negative geranium oil control; and (**B**) GC-MS selected ion chromatograms of the same ions for a negative geranium oil spiked at 0.1 ppm MHA. Note the absence of ions at *m/z* 282 in the negative specimen and also the totally different ion ratios for the ions 296/240.

GOV-027862

Other laboratories confirmed/substantiated our findings.[13–16] Meanwhile two other reports by investigators, supported by USP Lab (a major manufacturer of MHA containing dietary supplements), reported the presence of MHA in pelargonium samples and oils acquired from China for their studies.[2,17] It was suggested[17] that in order to address this controversy, a multi-centre study is recommended.

This report was initiated to determine if there is any credibility to the reports that MHA is a natural constituent of pelargonium (plant material or oil). Several samples (18 plant material and 9 oils) were acquired from different parts of the world for this investigation. These samples were split into four different subsets and each subset was subjected to analysis for MHA at a different laboratory, namely: ElSohly Laboratories, Inc.; The National Center for Natural Products Research; Shanghai Institute of Materia Medica; and The Second Military University School of Pharmacy. The identification of the samples used in the study and the results of testing at all four sites is shown in Table 1.

As can be seen from Table 1 none of the samples showed detectable levels of MHA at any of the analytical laboratories participating in this study.

As an example of the data generated at ElSohly Laboratories, Figure 5A shows the chromatograms for the two MRM's monitored for an unspiked *Pelargonium graveolens* plant sample from Yunnan Province, China, with no peaks at the R_t of MHA (i.e., negative for MHA), while Figure 5B shows the same sample spiked with 10 ng/mL MHA (proving recovery of MHA through the extraction method used), Figure 5C shows similar chromatograms for a calibrator sample at 10 ng/mL and Figure 5D shows a blank acetonitrile injection (showing same background peaks in the other chromatograms). The absence of either MRM peak in the chromatogram of the analyzed sample is typical in all samples analyzed. On the other hand, the recovery of MHA from a spiked sample shows that the absence of MHA peaks in the analyzed samples is because the compound is not there and not because of lack of extraction efficiency.

The data shown in Tables 2–5 represent the calibration curves (with instrument sensitivity), LOD and LOQ of the method, precision as well as recovery data carried out at the Second Military University, School of Pharmacy in Shanghai, China, Laboratory. Figure 6 shows representative chromatograms of plant material sample analyzed as is (showing no MHA) and chromatograms showing the same sample spiked with MHA (showing the MHA peaks), and a standard MHA chromatogram. Examples of chromatograms of *Pelargonium* oil samples as is and again spiked with MHA are provided in the supporting documents. It is clear that neither the plant materials, nor the oil samples showed MHA at the detection level of the method.

Analysis of the samples at the Shanghai Institute of Materia Medica again revealed that none of the samples had detectable levels of MHA. Chromatograms for samples 1 3 and oil sample 1 with no detectable signal at the R_t of the standard MHA are provided in the supporting documents.

Finally, Figure 7 shows examples of the LC-MS-TOF chromatograms for extracts of two oil samples as well as those of samples of young and mature leaves and one stem sample as compared to that of a control sample at 10 ng/mL, all analyzed at NCNPR. This analysis was intended to determine whether *Pelargonium* samples have any level of MHA that would produce an accurate mass corresponding to MHA. It is obvious from Figure 7 that, while the samples contain background noise that shows a mass of 116.10, that mass does not fit or agree with the accurate mass for MHA of 116.14, and therefore whatever the compound(s) is (are) at that retention time, it is not MHA.

While we were conducting this study and preparing this manuscript, a review on the issue of the presence or absence of MHA in *Pelargonium* appeared in *Analytical Chemistry Insights* by T. Gouthier.[18] The review attempted to distort the facts presented in our previous publication.[1] The author of the review overlayed the chromatogram of the ion at *m/z* 296 of the HFB derivative of a negative sample over the same ion chromatogram from a sample spiked with MHA from our GC-MS work, alleging the fact that the 'negative' sample showed a peak for that ion is an indication that that sample is actually not 'negative'. What is missing in the review is the fact that for GC-MS analysis we monitored three ions for the HFB-derivative of MHA, namely *m/z* 240, 296, and 282. In order for an unknown sample to be called positive for MHA, the sample not only has to have all three ions, but these ions should have ion ratios consistent with those of standard MHA, otherwise one *cannot* have a positive identification. The true picture of our previous work is shown in Figures 8A and 8B, where Figure 8A shows the three ion channels from the negative sample and Figure 8B shows the same ions from the negative sample spiked with MHA. Two major points are ignored by T. Gauthier in his review. First, the ion at *m/z* 282 is totally missing in the negative sample. This in itself, from a forensic standpoint and scientifically speaking, shows that whatever is giving peaks for the other ion(s) is not MHA. Furthermore, the ion ratios for the other ions at *m/z* 296 and 240 are quite different (ion ratio of 0.03) for MHA while the ratio in the negative sample is 0.24, again more proof that whatever is giving a peak at *m/z* 296 in the negative sample is NOT MHA. It is important to realize that the fact that there is a peak at the same retention time of MHA cannot be a reason to call the sample positive for MHA unless the peak satisfies the scientific and forensic requirements for a positive (i.e., all three ions are there and in the right ratio as those of the standard). Therefore, the data presented by T. Gauthier in his review[18] is misleading, erroneous and should be totally discounted.

It must be added here that reports continue to appear in the literature showing the negative and devastating side effects of the use of dietary supplements containing high levels of MHA. Karnatovskaia *et al.* reported cardiac arrest in a 21-year-old man after the ingestion of MHA containing a workout supplement.[19] Furthermore, Foley *et al.* reported on a series of acute liver injury cases, associated with the use of OxyELITE Pro (a supplement product containing MHA), with two of which requiring liver transplants.[20]

The danger of the use of products containing MHA is real, and healthcare professionals should be aware of the risks associated with consumption of dietary supplements containing MHA.

Conclusion

Twenty-seven different samples of *Pelargonium* plant material and oils from a avariety of sources were analyzed by four different laboratories. None of the laboratories found any MHA in any of the samples at the detection levels of the methods used. These results support previous reports that MHA found in dietary supplements is not of natural origin.

Acknowledgements

The authors thank Dr Yi Jin and Dr Jinghua Yang, School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan, China, for their help in sample collection and authentication and Prof. A.M. Viljoen, Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa, for providing samples from South Africa.

References

[1] M.A. ElSohly, W. Gul, K.M. ElSohly, T.P. Murphy, A. Weerasooriya, A.G. Chittiboyina, B. Avula, I. Khan, A. Eichner, L.D. Bowers. Pelargonium oil and methyl hexaneamine (MHA): Analytical approaches supporting the absence of MHA in authenticated *Pelargonium graveolens* plant material and oil. *J. Anal. Toxicol.* **2012,** *36*, 457.
[2] J.S. Li, M. Chen, Z.C. Li. Identification and quantification of dimethylamylamine in geranium by liquid chromatography tandem mass spectrometry. *Anal. Chem. Insights* **2012**, *7*, 47.
[3] E.E. Swanson, K.K. Chen. Comparison of pressor action of alicyclic derivatives of aliphatic amines. *J. Pharmacol. Exp. Ther.* **1948**, *93*, 423.
[4] D.F. Marsh. The comparative pharmacology of the isomeric heptylamines. *J. Pharmacol. Exp. Ther.* **1948**, *94*, 225.
[5] M. Lis-Balchin (Ed). Geranium and Pelargonium, in *Medicinal and Aromatic Plants- Industrial Profiles*. Taylor and Francis, New York, **2002,** *27*, 184.
[6] Z. Ping, Q. Jun, L. Qing. A study on the chemical constituents of geranium oil. *J. Guizhou Inst. Technol.* **1996**, *25*, 82.
[7] Classification of 1,3-Dimethylamylamine (DMAA), H.P.a.F.B. Health Canada, **2011**.
[8] WADA. The 2010 Prohibited List - International Standard. The World Anti-Doping Agency, **2010**.
[9] Unnamed Australians test positive for banned substance. BBC Sport, October 23, **2010**. http://news.bbc.co.uk/sport2/hi/front_page/9120842.stm [13 October 2014].
[10] P. Cossins. Rui Costa and his brother test positive. *Cycling News*, October 19, **2010**. http://www.cyclingnews.com/news/rui-costa-and-his-brother-test-positive [13 October 2014].
[11] P. Gee, S. Jackson, J. Easton. Another bitter pill: A case of toxicity from DMAA party pills. *N.Z. Med. J.* **2010**, *123*, 124.
[12] T.J. Tittern. Army probing connection between body building supplement, 2 deaths. *Stars and Stripes* **2011**. http://www.stripes.com/news/army-probing-connection-between-body-building-supplement-2-deaths-1.163652 [13 October 2014].
[13] Y. Zhang, R.M. Woods, Z.S. Breitbach, D.W. Armstrong. 1,3-Dimethylamylamine (DMAA) in supplements and geranium products: Natural or synthetic? *Drug Test. Anal.* **2012**, *12*, 986.
[14] C. Lorenzo, E. Moro, A. Santos, F. Uberti, P. Restani. Could 1,3 dimethylamylamine (DMAA) in food supplements have a natural origin? *Drug Test. Anal.* **2013**, *2*, 116.
[15] A. Lisi, N. Hasick, R. Kazlauskas, C. Goebel. Studies of methylhexaneamine in supplements and geranium oil. *Drug Test. Anal.* **2011**, *3*, 873.
[16] K.G. Austin, P.G. Travis, H.R. Leiberman. Analysis of 1,3 dimethylamylamine concentrations in Geraniacea, geranium oil and dietary supplements. *Drug Test. Anal.* **2014**, *7*, 797.
[17] H.L. Fleming, P.L. Ranaivo, P.S. Simone. Analysis and confirmation of 1,3-DMAA and 1,4-DMAA in geranium plants using high performance liquid chromatography with tandem mass spectrometry at ng/g concentrations. *Anal. Chem. Insights* **2012**, *7*, 59.
[18] D.G. Gauthier. Evidence for the presence of 1,3-Dimethylamylamine (1,3-DMAA) in geranium plant materials. *Anal. Chem. Insights* **2013**, *8*, 29.
[19] L.V. Karnatovskaia, J.C. Leoni, L. Freeman. Cardiac arrest in a 21 year old man after ingestion of 1,3-DMMA-containing workout supplements. *Clin. J. Sport Med.* **2014**, 1. DOI: 10.1097/JSM.0000000000000103
[20] S. Foley, E. Butlin, W. Shields, B. Lacey. Experience with OxyElite Pro and acute liver injury in active duty service members. *Dig. Dis. Sci.* **2014**, DOI: 10.1007/s10620-014-3221-4

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site.

Exhibit 26

Brandi Johnson

From: Ikhlas Khan [ikhan@olemiss.edu]
Sent: Wednesday, April 03, 2013 9:23 PM
To: Mahmoud A. ElSohly; Mahmoud A. Elsohly
Subject: FW: Pelargonium data
Attachments: Pelargonium plant material (guo).docx

From: ymn77 <ymn77@163.com>
Date: Thu, 4 Apr 2013 08:36:05 +0800
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "gda5958@163.com" <gda5958@163.com>
Subject: Re:Re: Pelargonium data

Dear Prof.
I checked the data I have and found that 2ng/ml DMAA in MeOH (control solution) could be detected by MRM method. The SNR was 38.3. The control solution was diluted from the 1mg/ml stock solution obtained from your lab.To evaluate the matrix effects, 100μl 2ng/ml DMAA was added to 100μl S1 (S2) sample. It was found that the peak area of the mix was approximately double of that of S1 (S2) (SNR>3). Thus, I think that 2ng/ml could be detected in the samples. The results were different from the report from Prof. Zhang's Lab, maybe due to the different amount of materials used in the experiment. Ten grams of S1 and S2 were used for analysis. Please find the detailed information in the attached document

--
Best regards,

Min Yang

National Engineering Laboratory for TCM Standardization Technology,
Shanghai Institute of Materia Medica.

At 2013-04-03 04:35:04,"Ikhlas Khan" <ikhan@olemiss.edu> wrote:

Dear Min
You have found 2 ng in some samples but It does not match with the report from Prof. Wei Dong's Lab. I think 2 ng is under detection limit, how did you find in these samples. Please confirm the results one more time. Our detection limit has been 10 ng if you say that we did not find anything under 10ng than we are Ok but if you find 2 ng, we need further confirmation.
Let me know if you have further question.
Appreciate your help.
IK

From: Ikhlas Khan <ikhan@olemiss.edu>
Date: Sun, 31 Mar 2013 21:39:59 -0500
To: ymn77 <ymn77@163.com>
Cc: "gda5958@163.com" <gda5958@163.com>
Subject: Re: Pelargonium data

Thanks
ik

From: ymn77 <ymn77@163.com>
Date: Mon, 1 Apr 2013 09:34:23 +0800
To: Ikhlas Khan <ikhan@olemiss.edu>

Cc: "gda5958@163.com" <gda5958@163.com>
Subject: Re:Fw:FW: Pelargonium data

Dear Prof. Khan
The two samples sent to Prof. Weidong Zhang from Kunming were listed in the table as S1 and S2. One sample sent to Prof. Guo was listed as S3. I gave all the infromation I know in the table. Attached file is the analysis detail of Pelargonium plant material and oil. All the analysis results and sample infromation could be found in the file.

--
Best regards,

Min Yang

National Engineering Laboratory for TCM Standardization Technology,
Shanghai Institute of Materia Medica.
在 2013-03-31 07:39:07，daguo@mail.shcnc.ac.cn 写道：

杨敏，
张卫东给的样品分析了没有？

-------- Forwarding messages --------
From: "Ikhlas Khan" <ikhan@olemiss.edu>
Date: 2013-03-31 05:18:27
To: "Dean Guo" <daguo@mail.shcnc.ac.cn>,zhangwei-dong <wdzhangy@hotmail.com>
Subject: FW: Pelargonium data
Dear
You got this email from Mahmoud. We send samples to you from Kunming and also you got some sample your own, we need full information to compile the results, which will get lot of attention during conference and would like to make sure everything is fine.

Any update who is coming or not coming?
IK

From: Waseem Gul <wgul@elsohly.com>
Date: Sat, 30 Mar 2013 14:55:29 -0500
To: <wdzhangy@hotmail.com>, Dean Guo <daguo@mail.shcnc.ac.cn>, Ikhlas Khan <ikhan@olemiss.edu>
Cc: "'Mahmoud A. ElSohly, Ph.D.'" <elsohly@elsohly.com>, Waseem Gul <wgul@elsohly.com>
Subject: Pelargonium data

We received the data on Pelargonium work from Juansu (through Dr. Khan on Jan.17th, 2013). We were expecting to get the data from both of your labs not only from the samples you collected but also from the two sets of samples we sent to you back in November 2012. The two set of samples were sent to you (Dr. Zang) and you were supposed to share with DeAn.

We are preparing a presentation for conference and we really need your data.

Thank you for a response to my request.

Mahmoud

Mahmoud A. ElSohly, Ph.D., BCFE, BCFM
President
ElSohly Laboratories, Incorporated (ELI)

5 Industrial Park Drive
Oxford, MS 38655
Tel (662) 236-2609
Fax (662) 234-0253
www.elsohly.com

Examination of 1,3-dimethylpentylamine (DMAA) in Pelargonium plant material

(Shanghai Institute of Materia Medica)
Min Yang, Dean Guo

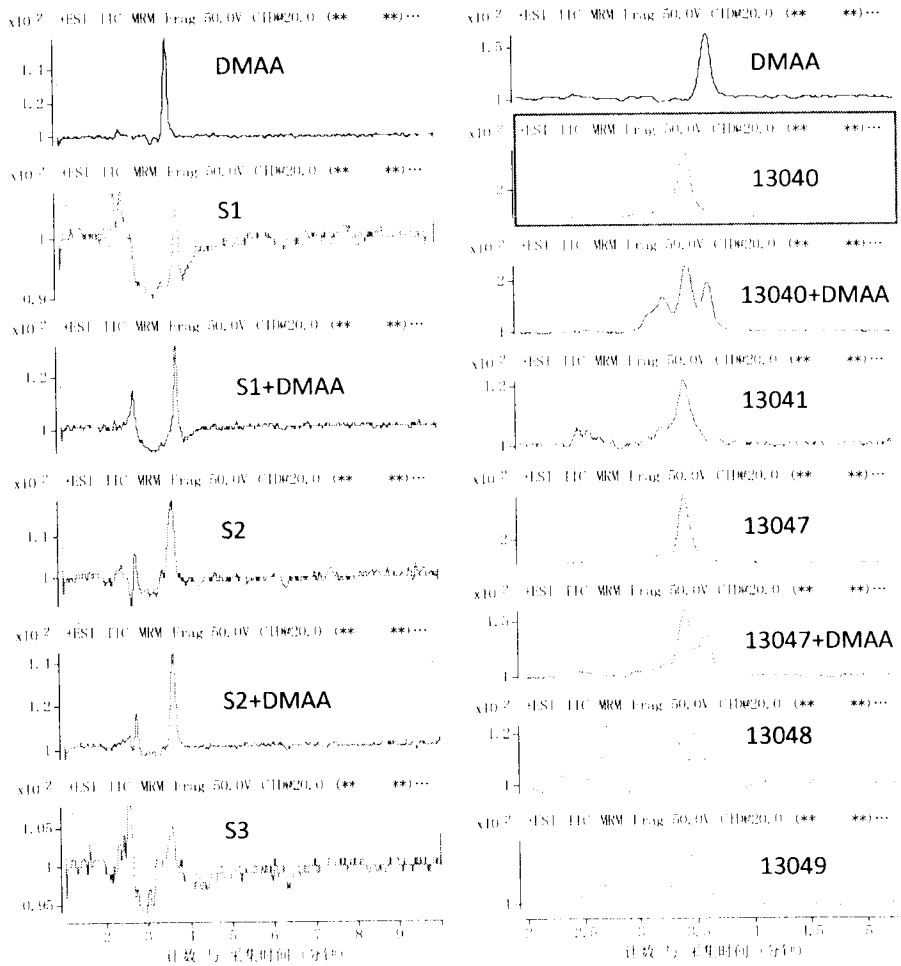
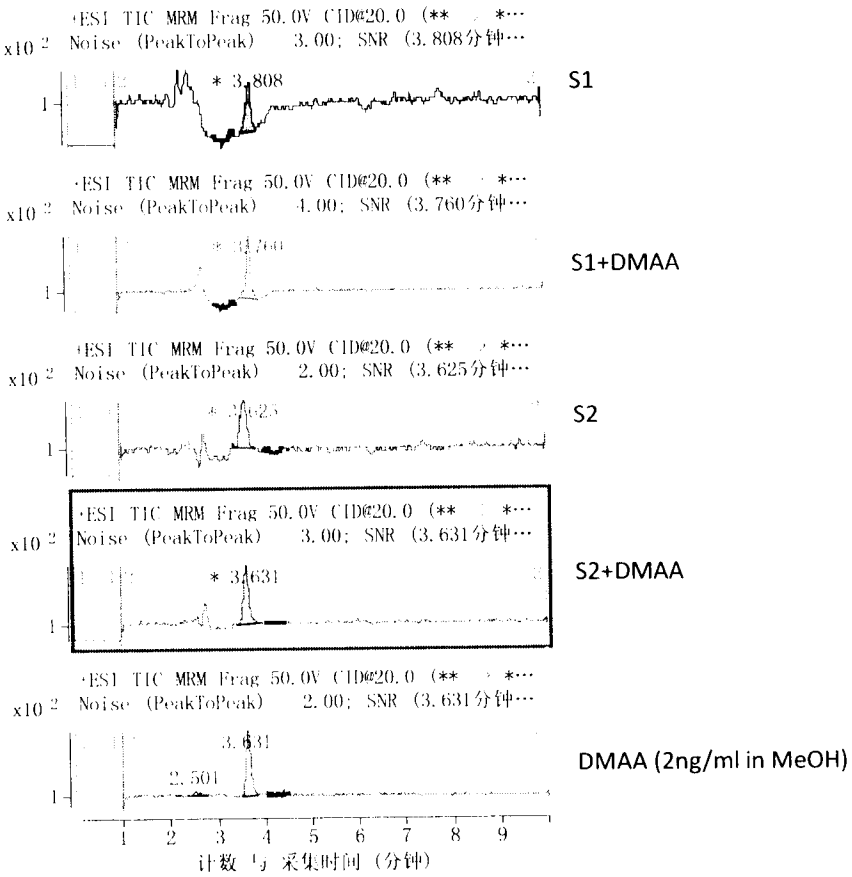


Figure 5. MRM chromatograms of some plant samples. 1,3-Dimethylpentylamine was detected in S1 and S2. The isomer of 1,3-Dimethylpentylamine was detected in 13040, 13041, 13047, 13048 and 13049.

Sample_ID	Genus	Species	Family	Part	Form	Amount	Content	Area
13040	Pelargonium	zonale cv 'Daredevil Salmon'	Geraniaceae	Leaf	Whole	0.3 g		
13041	Pelargonium	graveolens cv 'Bontrosai'	Geraniaceae	Stem	Whole	0.4 g		
13047	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Leaf		1 g		
13048	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Stem/Root		1 g		
13049	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Flower		1 g		
S1	Pelargonium	*zonale* Aif	Geraniaceae	Aerial part		10g	<2ng/ml	176

Sample_ID	Genus	Species	Family	Part	Form	Amount	Content	Area
S2	Pelargonium	*graveolens* L'Her	Geraniaceae	Aerial part		10g	<2ng/ml	102
S3	Pelargonium		Geraniaceae	Aerial part		5g		
DMAA							2ng/ml	443



Sample	RT	Area	Height	Peak Width	SNR
S1	3.808	107	12	0.319	4.1
S1+DMAA	3.76	233	35	0.407	8.9
S2	3.625	188	19	0.482	9.4
S2+DMAA	3.631	368	44	0.523	14.8
DMAA(2ng/ml in MeOH)	3.631	567	77	0.112	38.3

The data in table indicated that 2ng/ml DMAA in MeOH could be detected by MRM method. SNR was 38.3. To evaluate the matrix effects, 100µl 2ng/ml DMAA was added to 100µl S1 (S2) sample. It was found that the peak area of the mix was approximately double of that of S1 (S2) (SNR>3). Thus, I think that 2ng/ml could be detected in the samples. The results were different from the report from Prof. Zhang's Lab, maybe due to the different amount of material used in the experiment. Ten grams of S1 and S2 were used for analysis.

Methods

Control solution

The 1,3-Dimethylpentylamine solution was diluted to 2 ng/ml with methanol for anaylsis.

Extraction procedure

Sample 1 and 2 (S1 and S2): The aerial parts of fresh materials were cut to pieces and grounded with a mortar. Ten gram of samples was extracted with 50 ml 0.5 M HCl by sonication at 50 °C for 1 hour. The solution was filtered and adjusted to pH 9~10 using 10 N NaOH, extracted with dichloromethane (DCM). The DCM layer was evaporated and 1 ml MeOH was added to the residue and vortexed. The methanol solution was then transferred to an autosampler vial for analysis on the LC-MS/MS system.

LC-MS/MS system

The analysis was performed on an Agilent 1200 HPLC coupled to an Agilent 6410 Triple-Quadrupole mass spectrometer equipped with a JetStream™ ESI source (Agilent Technologies, Inc., Santa Clara, CA, USA). Chromatographic separation was performed on a Zorbax SB 150 mm × 4.6 mm C18 column (3.5 µm particles). The column temperature was the same as room temperature. The autosampler was fitted with a 20 µL injection loop. The injection volume was 2.0 µl for control and samples. The mobile phase A was 0.1% FA in MilliQ water and mobile phase B was 0.1% FA in acetonitrile (A:B=15:85). The flow rate was 0.6 mL/min. The total run time was 10 min. The retention time of DMAA was 3.97 min. The mass spectrometer was operated in positive ESI mode. The drying gas temperature and the flow rate were 350 °C and 8 L/min, respectively, and the nebulizer gas pressure was 45 psi. The capillary voltage was 4000 V. The mass spectrometer was operated in MRM mode at m/z 116.2 $[M+H]^+ \rightarrow$ 57.1 (quantification) and m/z 116.2 → 41.2 (qualification) for DMAA. The fragmentor energy was 50 V and collision energy was 20 eV. Both quadrupoles mass resolution were set to 2.5 units, respectively, and the dwell times were 200 ms for each *m/z* channel. Instrument control, data acquisition and quantification were performed by MassHunter Workstation software B.03.01 (Agilent Technologies, Torrance, USA).

Exhibit 27

A multi center study showing the absence of DMAA in Pelargonium

Mahmoud A. ElSohly, Waseem Gul, Kareem ElSohly, and Tim Murphy
ElSohly Labs and PSI, Oxford MS USA

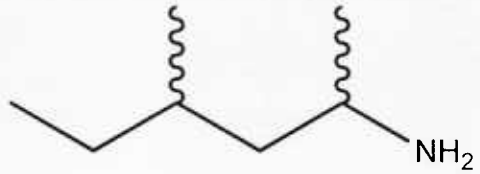
Ikhlas Khan, Bharathi Avula, Amar Chittiboyina and Troy Smillie
NCNPR, University of Mississippi, University MS USA

Wei-Dong Zhang and Juan Su
School of Pharmacy, Second Military Medical University, Shanghai China

Dean Guo and Min Yang
Shanghai Institute of Materia Medica, Shanghai China

PSI
PHYTOCHEMICAL SERVICES INCORPORATED

eLLi



1,3-Dimethyl amyl amine (DMAA) (**1**)
OR
1,3-Dimethyl pentylamine DMP
OR
2-Amino-4-methyl-hexane
OR
4-Methyl-2-hexylamine (MHA)

- Chemically, DMMA (CAS 105-41-9) is a simple aliphatic amine.
- DMAA has a number of chemical names including dimethylamylamine, 1,3-dimethylpentylamine, 2-amino-4-methylhexane, and 4-methyl-2-hexylamine.
- an α_1-adrenergic agonist, and was shown to be two hundred-fold less potent than *l*-epinephrine as a vasopressor in dogs but with a much longer duration of action
- an active ingredient in party pills in New Zealand, where it has replaced 1-benzylpiperazine.
- Due to its purported stimulant effects and health risks the Canadian Ministry of Health has clarified that under their regulatory system MHA is a drug.

DMAA

- Dimethylamylamine is a drug made synthetically in a laboratory.
- Originally used as a nasal decongestant.
- Sold as a dietary supplement for weight loss.
- Used by many athletes to improve performance and body building.
- In 2010, DMMA was added to the World Anti-Doping Agency's prohibited substances list.
- DMAA has resulted in a number of reported doping cases involving Indian, Nigerian, and US athletes, presumably due to consumption of dietary supplements containing DMAA.
- Beginning in December of 2011, following the deaths of 2 soldiers who used supplements with DMAA, the US military removed all such supplements from military exchanges around the world.

- After four adverse events requiring emergency care, the New Zealand Health Ministry is also considering officially scheduling the drug.
- In the United States (US) and elsewhere, DMAA is increasingly being found in nutritional supplements such as in weight loss and exercise 'stimulant' supplements.
- In many cases, the product listed geranium oil or some part of the geranium plant on the content label, and in a few cases the label listed the main ingredient as DMAA.
- Synthetic DMAA can also be purchased in bulk from several chemical suppliers, and can be purchased over the internet.
- The inclusion of DMAA in dietary supplements was based on a report of its being a constituent of pelargonium oil (0.6%)

(Ping, Z., Q. Jun, and L. Qing: A study on the chemical constituents of geranium oil. *Journal of Guizhou Institute of Technology* **25**: 82-85:(1996))

- Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), dietary supplements in the US that contain herbs and other botanicals as well as their constituents or extracts or concentrates, may be legally sold as (or in) dietary supplements.

- Therefore, if DMAA is detectable in *P. graveolens* (or any other plant) AND it is extracted from that plant and added to the supplement (as opposed to being synthesized) then it may be legally sold in dietary supplements.

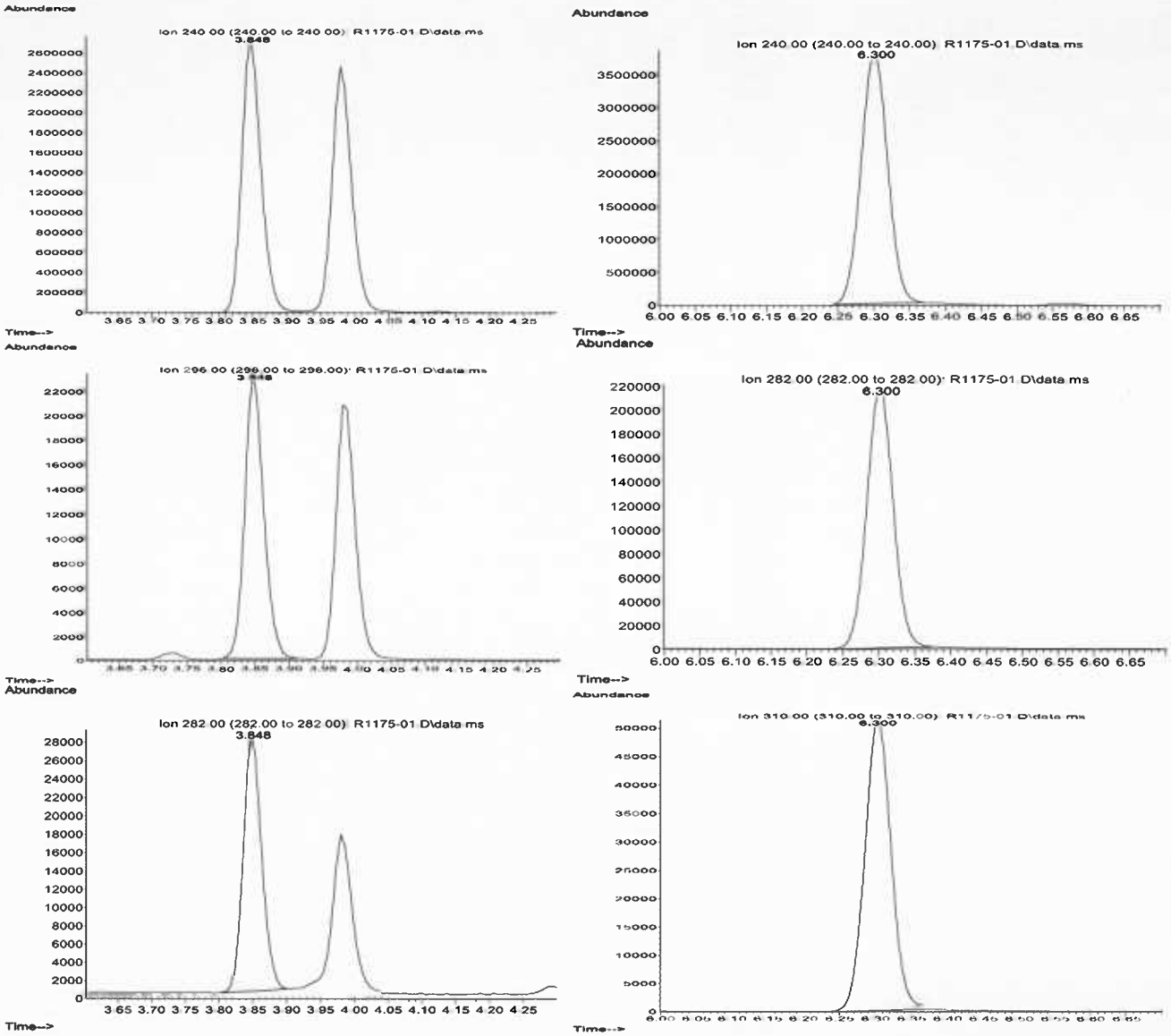
- On the other hand, substances that do not meet the definition of a dietary supplement set forth in DSHEA must go through the US FDA's New Dietary Ingredient (NDI) notification process.

- The latter process requires the manufacturer to submit data to support safety of the compound under the conditions of use.

- Given DMAA is a substance prohibited in many sports, and is a stimulant that reportedly carries significant health risks, it was crucial to determine whether DMAA could indeed be detected in *P. graveolens* plant material or oil.

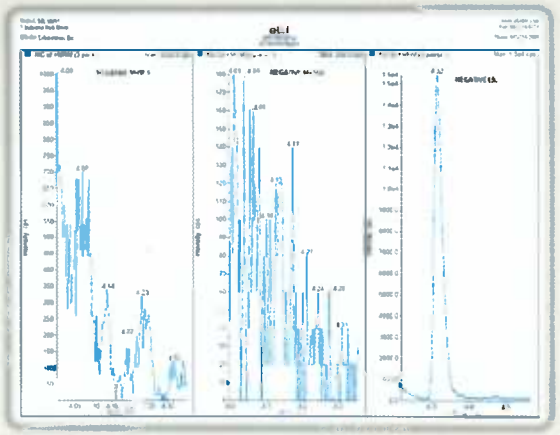
In 2011 We Initiated A Study to:

- Acquire Authenticated *Pelargonium* Plant Material (15 samples).
- Acquire Authenticated *Pelargonium* Oil Material (2 samples).
- Procure A variety of Commercial *Pelargonium* Oils (19 samples) and dietary supplements label to contain *pelargonium* as the source of DMAA or just DMA (3).
- Develop and validate GC/MS and LC/MS methods for DMAA.
- Analyze All samples to establish presence /or absence of DMAA.

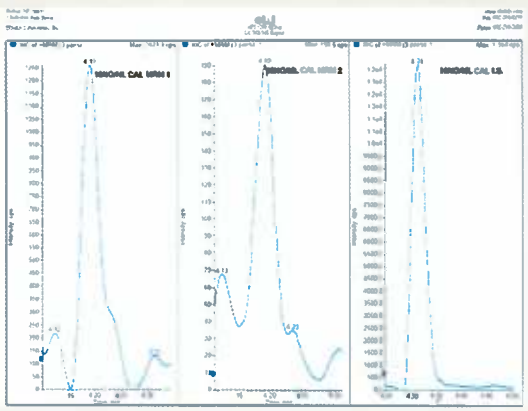


GC/MS selected ion chromatograms for MHA as the heptafluorobutyrate (HFB) derivative, (1 µg unextracted standard) with I.S. MHA ions at *m/z* 240,296, and 282 (top to bottom) are shown on the left, while those of the I.S.-HFB (*m/z* 240,282, and 310) are shown on the right.

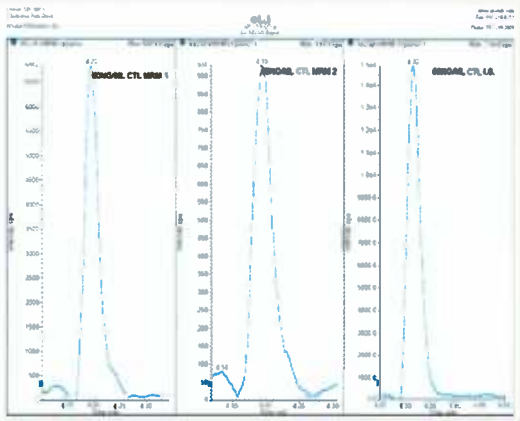
A



B



C



D

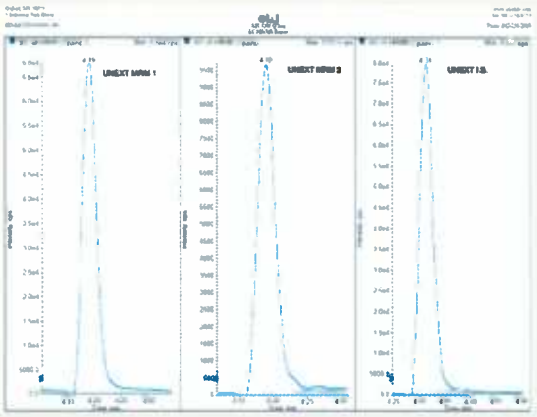


Figure 1: Chromatograms for MRM 1, MRM 2 and I.S. of negative (A), 10ng/ml calibrator (B), 50ng/ml control (C), and un-extracted MHA and I.S. at 50ng/ml (D)

Concentration of DMAA in samples of the authenticated volatile oil of *P. graveolens*, authenticated plant material (leaves and stems) as well as several commercial oils and three products alleged to contain DMAA as a component of *P. graveolens* oil.

Authentic Plant Material			
Sample #	**Sample Name**	**GC-MS Conc. of DMAA**	**LC-MS-MS Conc. of DMAA**
PSI-5	*Pelargonium graveolens* (Geranium), dried plant	ND	ND*
PSI-54-3A	Dried stem A	NA**	ND
PSI-54-3B	Dried stem B	NA	ND
PSI-54-3C	Fresh stem A	NA	ND
PSI-54-3D	Fresh stem B	NA	ND
PSI-54-1A	Dried mature leaves A	NA	ND
PSI-54-1B	Dried mature leaves B	NA	ND
PSI-54-1C	Fresh mature leaves A		ND
PSI-54-1D	Fresh mature leaves B	NA	ND
PSI-54-2A	Dried young leaves A	NA	ND
PSI-54-2B	Dried young leaves B	NA	ND
PSI-54-2C	Fresh young leaves A	NA	ND
PSI-54-2D	Fresh young leaves B	NA	ND

Authentic Volatile Oils of *P. graveolens*			
EOIL-14	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-15	*Pelargonium graveolens* (Geranium)	ND	ND
	UM Oil		ND
Commercial Volatile Oils			
EOIL-1	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-2	*Pelargonium graveolens*(Geranium)	ND	ND
EOIL-3	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-4	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-5	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-6	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-7	*Pelargonium graveolens* (Geranium Bourbon)	ND	ND
EOIL-8	*Pelargonium graveolens* (Geranium Egyptian)	ND	ND
EOIL-9	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-10	*Pelargonium graveolens* (Geranium)	ND	ND
EOIL-11	Wild Geranium; Herb Pharma	ND	ND
EOIL-16	*Pelargonium odorantissimum*	ND	ND
PSI-18	Geranium Essential Oil	ND	ND
PSI-20-1	Organic vegetal extract oil	ND	ND
PSI-20-2	Geranium Oil	ND	ND
PSI-20-3	*Pelargonium Odorantissimum* (Geranium oil)	ND	ND
PSI-20-4	Geranium oil	ND	ND
PSI-20-5	Geranium oil	ND	ND
PSI-22	100% pure geranium	ND	ND
PSI-25	100% geranium	ND	ND
Products other than Volatile Oil			
EOIL-17	Product B	> 10 mg/g	> 10 mg/g
EOIL-18	Product A	> 2 mg/g	> 2 mg/g
PSI-17-2	Product C		< 1 mg/g

* ND = Non detected (below LOD of 10 ng/ml or 10 ng/g), NA** = Not analyzed

We therefore concluded:

- DMAA is **NOT** A Component of *Pelargonium* Plant Material or Oil
- The high levels of DMAA in Commercial Dietary Supplements Must be of Synthetic Origin
- Simple mathematics make it impossible for those levels to be of Natural origin (If we assume that a dietary supplement contains 10 mg DMAA per capsule, then one bottle of 100 capsules would require the processing of 167 kg of *P. graveolens* leaves).
- Results were published in JAT in 2012.

***Pelargonium* Oil and Methyl Hexaneamine (MHA): Analytical Approaches Supporting the Absence of MHA in Authenticated Pelargonium graveolens Plant Material and Oil**

ElSohly, Mahmoud A.; Gul, Waseem; ElSohly, Kareem M.; Murphy, Timothy P.; Weerasooriya, Aroona; Chittiboyina, Amar G.; Avula, Bharathi; Khan, Ikhlas; Eichner, Amy; Bowers, Larry D. Journal of Analytical Toxicology (2012), 36(7), 457-471.

Other publications supporting our findings:

Zhang, Y., Woods, R.M., Breitbach, Z.S. and Armstrong, D.W. (2012). 1,3-Dimethylamylamine (DMAA) in supplements and geranium products: natural or synthetic?
***Drug Testing and Analysis*. DOI: 10.1002/dta.1368**

No DMMA was detected at a level of ≥10 ppb in any of the 8 geranium samples.

Lorenzo C, Moro E, Santos A, Uberti F, and Restani P. (2012). Could 1,3 dimethylamylamine (DMAA) in food supplements have a natural origion?
***Drug Testing and Analysis*. DOI10.1002/dta.1391**

DMAA was not found in any of the leaves or stems in 4 geranium species and 3 well known cultivars or in commercial geranium oils investigated.

FDA NEWS RELEASE
For Immediate Release: April 27, 2012
Media Inquiries: Tamara Ward, 301-796-7567, tamara.ward@fda.hhs.gov
Trade Press Inquiries: Sebastian Cianci, 240-402-2291, sebastian.cianci@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

FDA challenges marketing of DMAA products for lack of safety evidence

Agency cites ten companies in warning letters

The U.S. Food and Drug Administration today issued warning letters to ten manufacturers and distributors of dietary supplements containing dimethylamylamine, more popularly known as DMAA, for marketing products for which evidence of the safety of the product had not been submitted to FDA.

Also referred to as 1,3-dimethylamylamine, methylhexanamine, or geranium extract, the ingredient is in dietary supplements and is often touted as a "natural" stimulant.

"Before marketing products containing DMAA, manufacturers and distributors have a responsibility under the law to provide evidence of the safety of their products. They haven't done that and that makes the products adulterated," said Daniel Fabricant, Ph.D., Director of FDA's Dietary Supplement Program.

Specifically, the warning letters cite the companies for marketing products for which a notification had not been submitted for the use of DMAA as a New Dietary Ingredient (NDI). Under current law, dietary supplement manufacturers or distributors who use certain dietary ingredients not marketed in a dietary supplement prior to October 15, 1994, are responsible for notifying the FDA of evidence to support their conclusion that their dietary supplements containing NDIs are safe. Manufacturers or distributors must submit notification at least 75 days before marketing their products. The companies warned today were marketing products for which this requirement had not been met.

The FDA warning letters also advised the companies that the agency is not aware of evidence or history of use to indicate that DMAA is safe. Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), manufacturers, marketers and distributors of dietary supplements are responsible for ensuring that they are marketing a safe product.

The FDA letters noted that DMAA is known to narrow the blood vessels and arteries, which can elevate blood pressure and may lead to cardiovascular events ranging from shortness of breath and tightening in the chest to heart attack. **The agency has received 42 adverse event reports on products containing DMAA. While the complaints do not establish that DMAA was the cause of the incidents, some of the reports have included cardiac disorders, nervous system disorders, psychiatric disorders, and death.**

The agency additionally warned the companies that synthetically-produced DMAA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement. DSHEA defines a dietary ingredient as a vitamin, mineral, amino acid, herb or other botanical, a dietary substance for use by man to supplement the diet, or a concentrate, metabolite, constituent, extract, or combination of these substances.

The companies have 15 business days to respond to the FDA with the specific steps they will take to address the issues in the warning letters.

The companies receiving warning letters and their product names are:

Company	Product(s)
Exclusive Supplements[1]	Biorhythm SSIN Juice
Fahrenheit Nutrition[2]	Lean Efx
Gaspari Nutrition[3]	Spirodex
iSatori Global Technologies, LLC[4]	PWR
Muscle Warfare, Inc.[5]	Napalm
MuscleMeds Performance Technologies[6]	Code Red
Nutrex Research[7]	Hemo Rage Black Lipo-6 Black Ultra Concentrate Lipo-6 Black Lipo-6 Black Hers Ultra Concentrate Lipo-6 Black Hers
SEI Pharmaceuticals[8]	MethylHex 4,2
SNI LLC[9]	Nitric Blast
USP Labs, LLC[10]	Oxy Elite Pro Jack3D

Analytical work published in 2012 Alledging high amounts of DMAA in *Pelargonium* Plant and Oils

Li, J.S., Chen, M., and Li, Z.C. (2012). Identification and quantification of dimethylamylamine in geranium by liquid chromatography tandem mass spectrometry. *Analytical Chemistry Insights* 7: 47-58. DOI: 10.4137/ACI.S9969
Plants were provided by Dr. Yi Jin from different parts of China (Yunan, Jiangsu and Guizhou). Oils were from Jianxi province).

Fleming, H.L., Ranaivo, P.L., and Simone, P.S. (2012). Analysis and confirmation of 1,3-DMAA and 1,4-DMAA in geranium plants using high performance liquid chromatography with tandem mass spectrometry at ng/g concentrations. *Analytical Chemistry Insights*. 7: 59-78. DOI: 10.4137/ACI.S10445
Samples were from Changzhou, Guiyang, and Kunming provinces in China.

Both supported by USPLab the manufacturer of dietary supplements with high levels of DMAA and both studies published in a open access journal.

http://www.nutraingredients-usa.com/Research/USPlabs-funded-study-claims-to-confirm-presence-of-DMAA-in-Chinese-geranium

Levels of 1,3-DMAA and 1,4-DMAA in geranium (*Pelargonium graveolens*) and geranium oil from different sources.

Sample ID	Source	Date of collection	1,3-DMAA (ng/g)	1,4-DMAA (ng/g)
070611-0164 (plant)	Yunnan, China	June 9, 2011	**13.60**	**3.56**
072811-1026 (plant)	Jiangsu, China	June 9, 2011	**165.0**	**35.30**
072811-1027 (plant)	Guizhou, China	June 5, 2011	**365.0**	**9.12**
051911-0588 (oil)	Jiangxi, China	-	**13271**	**220.0**
042911-0988 (oil)	Jiangxi, China	-	**167.0**	Not detected
042911-0989 (oil)	Jiangxi, China	-	**377.0**	Not detected

Li, J.S., Chen, M., and Li, Z.C. (2012). Identification and quantification of dimethylamylamine in geranium by liquid chromatography tandem mass spectrometry. *Analytical Chemistry Insights* 7: 47-58. DOI: 10.4137/ACI.S9969

Levels of 1,3-DMAA and 1,4-DMAA in geranium (*Pelargonium graveolens*) and geranium oil from different sources.

Sample ID	Source	Date of collection	1,3-DMAA (ng/g)	1,4-DMAA (ng/g)
Pelargonium graveolens	Kunming, China	June 2011	-	-
	Guiyang, China	March 2012	-	-
	Changzhou, China*	May 2012	**68-496** ng/g	**13-162** ng/g

*Same sample analyzed be Li *et al*.

Fleming, H.L., Ranaivo, P.L., and Simone, P.S. (2012). Analysis and confirmation of 1,3-DMAA and 1,4-DMAA in geranium plants using high performance liquid chromatography with tandem mass spectrometry at ng/g concentrations. *Analytical Chemistry Insights*. 7: 59-78. DOI: 10.4137/ACI.S10445

In this current study:

- Multiple samples were collected of plant material and oils from around the world.
- Samples were submitted to four sites for analysis.
- All sites adopted the same extraction method reported by Li *et. al* (2012).
- All sites used LC/MS/MS or LC/MS-ToF.
- Detection limit set at 10ng/mL as previously reported by ElSohly *et al*.

#.	Samples	Specimen
1	Sample #1	*Pelargonium zonale* Aif, from Yunnan province, China
2	Sample #2	*Pelargonium graveolens* L' Her, Yunnan province, China
3	Sample #3	Provided by De' an Guo Yunnan province, China
4	No 1: (little bottle)	Ni de lan Rose Geranium oil Provided by Fangli Biotechnology limited company Kunming, Yunnan province
5	No 2: (little bottle)	Geranium oil
6	No 3: (little bottle)	Geranium oil-2012
7	No 4: (little bottle)	Geranium oil- no label
8	1286 (Egypt)	NCNPR# 13151
9	1653 (Gingind lovu)	NCNPR# 13152
10	1758 (Ntsimbini)	NCNPR# 13153
11	1759 (Nelspruit)	NCNPR# 13154
12	1787 (Kristammahoek)	NCNPR# 13155
13	*Pelargonium zonale* cv' daredevvk salmon' (stem)	NCNPR# 13039
14	*Pelargonium zonale* cv' daredevvk salmon' (leaf)	NCNPR# 13040
15	*Pelargonium graveolens* cv 'Bontrosai' (stem)	NCNPR# 13041

#	Samples	Specimen
16	*Pelargonium graveolens* cv 'Bontrosai' (root)	NCNPR# 13042
17	*Pelargonium graveolens* cv 'Bontrosai' (leaf)	NCNPR# 13043
18	*Pelargonium graveolens* (leaf)	NCNPR #13044
19	*Pelargonium graveolens* (stem)	NCNPR #13045
20	*Pelargonium tomentosum* (leaf)	NCNPR# 13046
21	*Pelargonium hortorum* (leaf)	NCNPR# 13047
22	*Pelargonium hortorum* (stem/root)	NCNPR# 13048
23	*Pelargonium hortorum* (flower)	NCNPR# 13049
24	*Pelargonium odoratissimum* (whole plant)	NCNPR# 10591
25	*Pelargonium tomentosum* (stem)	NCNPR# 10605
26	*Pelargonium hortorum* (whole plant)	NCNPR# 10616
27	*Pelargonium denticulatum* (leaf)	NCNPR# 10636

Pelargonium graveolens:

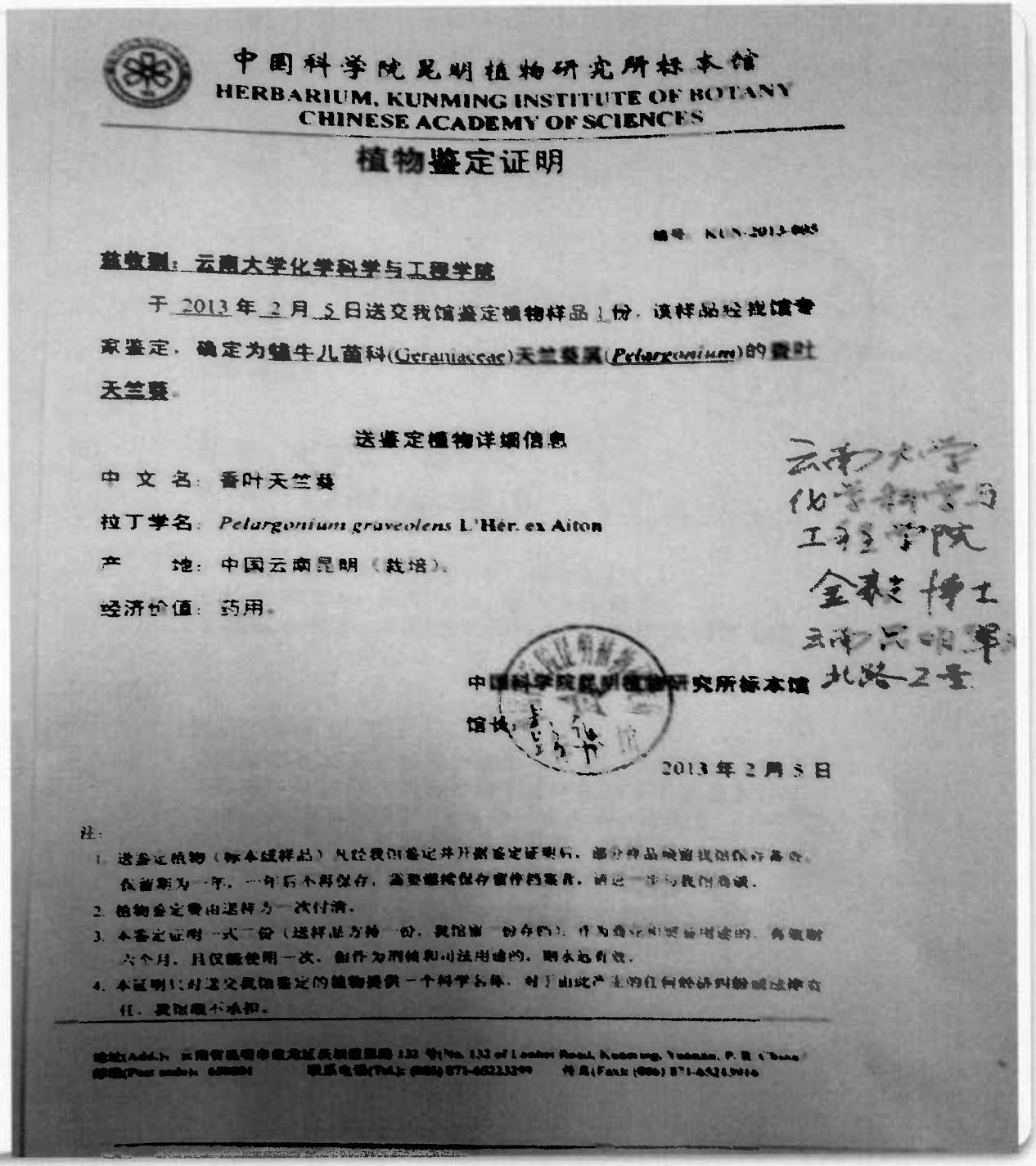
The plant material was provided by:

Dr. Yi Jin

School of Chemical Science and Technology
Yunnan University,
Kunming, Yunnan, China

The authentication was performed by:

Herbarium, Kunming Institute of Botany Chinese Academy of Science, Kunming, Yunnan, China



中国科学院昆明植物研究所标本馆
HERBARIUM, KUNMING INSTITUTE OF BOTANY
CHINESE ACADEMY OF SCIENCES

植物鉴定证明

编号: KUN-2013-005

兹收到：云南大学化学科学与工程学院

于 2013 年 2 月 5 日送交我馆鉴定植物样品 1 份，该样品经我馆专家鉴定，确定为蝶形花科(Geraniaceae)天竺葵属(*Pelargonium*)的香叶天竺葵。

送鉴定植物详细信息

中 文 名：香叶天竺葵

拉丁学名：*Pelargonium graveolens* L'Hér. ex Aiton

产　　地：中国云南昆明（栽培）。

经济价值：药用。

云南大学
化学科学与
工程学院
金教师
云南昆明
北路2号

中国科学院昆明植物研究所标本馆
馆长：
2013 年 2 月 5 日

注：

1. 送鉴定植物（标本或样品）凡经我馆鉴定并开据鉴定证明后，部分样品或留我馆保存备查，保留期为一年，一年后不再保存，需要继续保存请作档案者，请进一步与我馆商谈。
2. 植物鉴定费由送样方一次付清。
3. 本鉴定证明一式二份（送样单方持一份，我馆留一份存档），作为鉴定结果备用凭证的，有效期六个月，且仅能使用一次，创作为刑侦和司法用途的，则永远有效。
4. 本证明只对送交我馆鉴定的植物提供一个科学名称，对于由此产生的任何经济纠纷或法律责任，我馆概不承担。

地址(Add.): 云南省昆明市北京路132号(No. 132 of Lanhei Road, Kunming, Yunnan, P. R. China)
邮编(Post code): 650204　　联系电话(Tel.): (0086) 871-65223249　　传真(Fax): (0086) 871-65215916

Pelargonium zonale Aif

The plant material was purchased from:

Botanical Garden of Haigeng State Park, Kunming, Yunnan, China

The authentication was performed by:

Biochemistry Department, School of Life Science Yunnan University, Kunming, Yunnan, China

Essential Oils:

Pelargonium graveolens essential oil (**sample on the right**)

The essential oil was provided by:
Dr. Yi Jin
School of Chemical Science and Technology
Yunnan University, Kunming, Yunnan, China

Pelargonium graveolens essential oil (**sample on the left**)
The essential oil was purchased from:
Shangyi Flower Market,
Kunming, Yunnan, China

Pelargonium graveolens essential oil

The essential oil was provided by:
Perfume Research and Development Center,
Yunnan Agriculture University
Kunming, Yunnan, China

Dr. Jinghua Yang
School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan, China, also helped in sample collection and authentication.

Examination of 1,3-dimethylpentylamine (DMAA) in Pelargonium plant material and oil

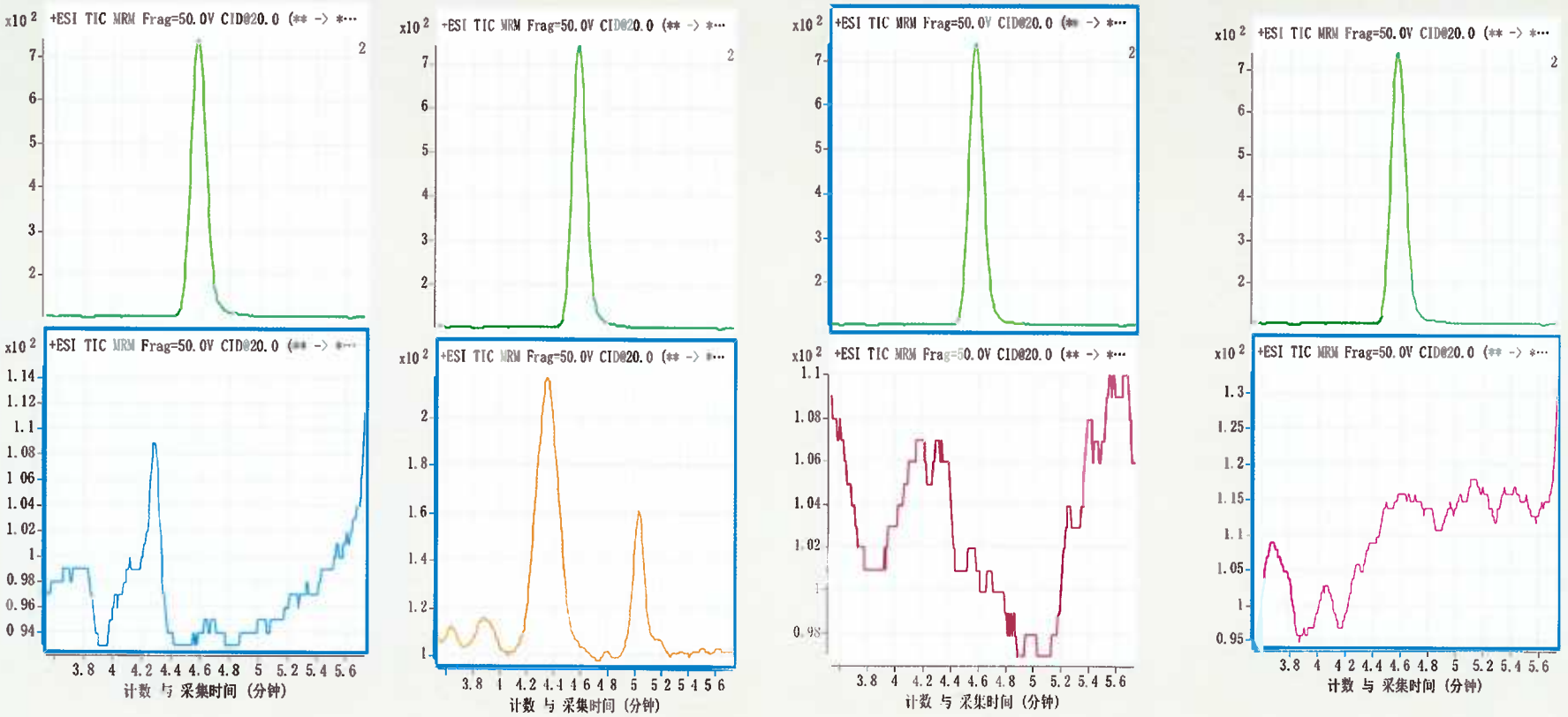
(Shanghai Institute of Materia Medica)
Min Yang, Dean Guo

LC-MS/MS

The analysis was performed on an *Agilent 1200 HPLC coupled to an Agilent 6410 Triple-Quadrupole mass spectrometer* equipped with a JetStream™ ESI source (Agilent Technologies, Inc., Santa Clara, CA, USA). Chromatographic separation was performed on a Zorbax SB 150 mm × 4.6 mm C18 column (3.5 μm particles). The column temperature was the same as room temperature. The autosampler was fitted with a 20 μL injection loop. The injection volume was 2.0 μl for control and samples. The mobile phase A was 0.1% FA in MilliQ water and mobile phase B was 0.1% FA in acetonitrile (A:B=15:85). The flow rate was 0.6 mL/min. The total run time was 10 min. The retention time of DMAA was 3.97 min. The mass spectrometer was operated in positive ESI mode. The drying gas temperature and the flow rate were 350 ˚C and 8 L/min, respectively, and the nebulizer gas pressure was 45 psi. The capillary voltage was 4000 V. The mass spectrometer was operated in MRM mode at m/z 116.2 $[M+H]^+ \rightarrow$ 57.1 (quantification) and m/z 116.2 → 41.2 (qualification) for DMAA. The fragmentor energy was 50 V and collision energy was 20 eV. Both quadrupoles mass resolution were set to 2.5 units, respectively, and the dwell times were 200 ms for each *m/z* channel. Instrument control, data acquisition and quantification were performed by MassHunter Workstation software B.03.01 (Agilent Technologies, Torrance, USA).

Sample_ID	Genus	Species	Family	Part	Form	Amount
10591	Pelargonium	odoratissimum	Geraniaceae	Whole plant	C/S	1 g
10605	Pelargonium	tomentosum	Geraniaceae	Stem	C/S	1 g
10616	Pelargonium	hortorum	Geraniaceae	Whole plant	C/S	1 g
10636	Pelargonium	denticulatum	Geraniaceae	Whole plant	C/S	1 g
13039	Pelargonium	zonale cv 'Daredevil Salmon'	Geraniaceae	Stem	Whole	1 g
13040	Pelargonium	zonale cv 'Daredevil Salmon'	Geraniaceae	Leaf	Whole	0.3 g
13041	Pelargonium	graveolens cv 'Bontrosai'	Geraniaceae	Stem	Whole	0.4 g
13042	Pelargonium	graveolens cv 'Bontrosai'	Geraniaceae	Root	Whole	1 g
13043	Pelargonium	graveolens cv 'Bontrosai'	Geraniaceae	Leaf	Whole	0.6 g
13044	Pelargonium	graveolens	Geraniaceae	Leaf		1 g
13045	Pelargonium	graveolens	Geraniaceae	Stem		1 g
13046	Pelargonium	tomentosum	Geraniaceae	Leaf		1 g
13047	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Leaf		1 g
13048	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Stem/Root		1 g
13049	Pelargonium	hortorum cv. 'F1 Freckles'	Geraniaceae	Flower		1 g
13151	Pelargonium	graveolens	Geraniaceae	Essential oil	Oil	200µl
13152	Pelargonium	graveolens	Geraniaceae	Essential oil	Oil	200µl
13153	Pelargonium	graveolens	Geraniaceae	Essential oil	Oil	200µl
13154	Pelargonium	graveolens	Geraniaceae	Essential oil	Oil	200µl
13155	Pelargonium	graveolens	Geraniaceae	Essential oil	Oil	200µl
S1	Pelargonium	zonale Aif	Geraniaceae	Aerial part		10g
S2	Pelargonium	graveolens L'Her	Geraniaceae	Aerial part		10g
S3	Pelargonium		Geraniaceae	Aerial part		5g
O1	Pelargonium		Geraniaceae	Essential oil		200µl
O2	Pelargonium	graveolens L'Her	Geraniaceae	Essential oil		200µl
O3	Pelargonium	graveolens L'Her	Geraniaceae	Essential oil		200µl
O4	Pelargonium	graveolens L'Her	Geraniaceae	Essential oil		200µl

None of them showed detectable level of DMAA.



MRM chromatograms of three plant samples (S1~S3) and one oil sample (O1) with control chromatogram (upper, 16 ng/ml DMAA).

Analysis at the University of Mississippi (NCNPR)-USA by LC-QTOF-MS

B. Avula and I. Khan

UHPLC	
UPLC	**Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA)**
Column	ACQUITY UPLC BEH C18 Column (2.1 x 50 mm, 1.7 µm, Waters)
Mobile Phase	Water and Acetonitrile, both containing formic acid
Temperature	40 °C
Flow Rate	0.25 mL/min
Run time	4 min
Injected Volume	10 µL

QTOF-MS	
MS	**Xevo™ QTOF-MS (Waters Corp., Manchester, UK)**
Ionization mode	ESI+
Source temperature	150 °C
Desolvation temperature	350 °C
Desolvation gas flow	900 L/h
Capillary voltage	3 kV
Cone voltage	30 V
Collision Energy	3 V
Lock mass compound	Leucine Enkephalin (*m/z* 556.2771)
Mass Accuracy	<10 ppm

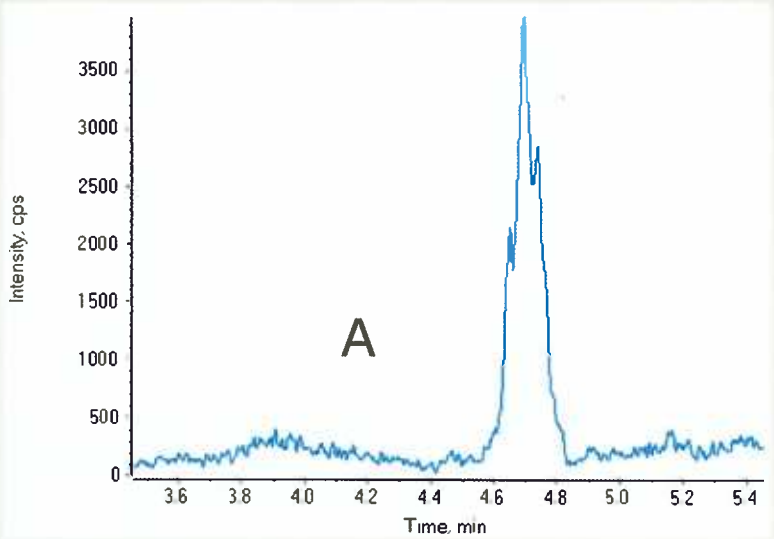
#.	Samples	Specimen	DMAA (m/z =116.1439→57.06)
1	**Sample #1**	***Pelargonium zonale* Aif, from Yunnan province, China**	**ND**
2	Sample #2	*Pelargonium graveolens* L' Her, Yunnan province, China	**ND**
3	Sample #3	Provided by De' an Guo Yunnan province, China	**ND**
4	No 1: (little bottle)	Ni de lan Rose Geranium oil Provided by Fangli Biotechnology limited company Kunming, Yunnan province	**ND**
5	No 2: (little bottle)	Geranium oil	**ND**
6	No 3: (little bottle)	Geranium oil-2012	**ND**
7	No 4: (little bottle)	Geranium oil- no label	**ND**
8	1286 (Egypt)	NCNPR# 13151	**ND**
9	1653 (Gingind lovu)	NCNPR# 13152	**ND**
10	1758 (Ntsimbini)	NCNPR# 13153	**ND**
11	1759 (Nelspruit)	NCNPR# 13154	**ND**
12	1787 (Kristammahoek)	NCNPR# 13155	**ND**
13	*Pelargonium zonale* cv' daredevvk salmon' (stem)	NCNPR# 13039	
14	*Pelargonium zonale* cv' daredevvk salmon' (leaf)	NCNPR# 13040	**ND**
15	*Pelargonium graveolens* cv 'Bontrosai' (stem)	NCNPR# 13041	**ND**

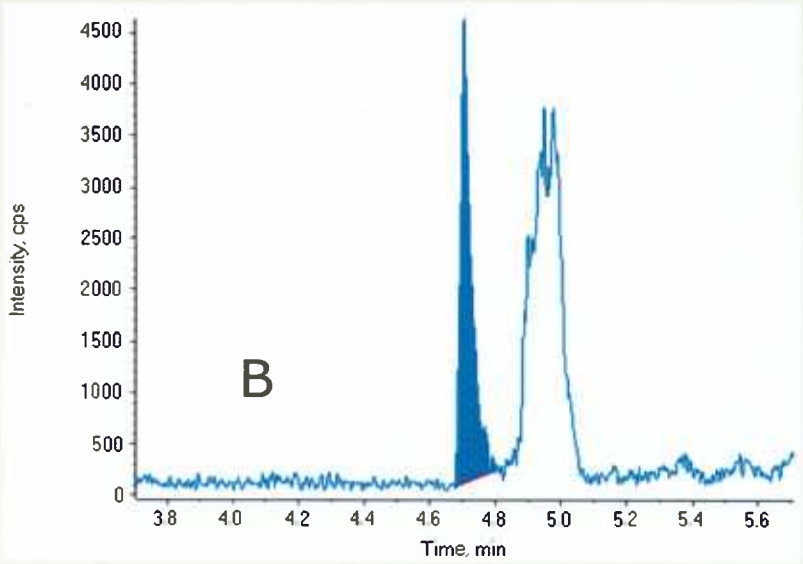
#	Samples	Specimen	DMAA (m/z =116.1439→57.06)
16	*Pelargonium graveolens* cv 'Bontrosai' (root)	NCNPR# 13042	ND
17	*Pelargonium graveolens* cv 'Bontrosai' (leaf)	NCNPR# 13043	ND
18	*Pelargonium graveolens* (leaf)	NCNPR #13044	ND
19	*Pelargonium graveolens* (stem)	NCNPR #13045	ND
20	*Pelargonium tomentosum* (leaf)	NCNPR# 13046	ND
21	*Pelargonium hortorum* (leaf)	NCNPR# 13047	ND
22	*Pelargonium hortorum* (stem/root)	NCNPR# 13048	ND
23	*Pelargonium hortorum* (flower)	NCNPR# 13049	ND
24	*Pelargonium odoratissimum* (whole plant)	NCNPR# 10591	ND
25	*Pelargonium tomentosum* (stem)	NCNPR# 10605	ND
26	*Pelargonium hortorum* (whole plant)	NCNPR# 10616	ND
27	*Pelargonium denticulatum* (leaf)	NCNPR# 10636	ND

Analysis at ElSohly Laboratories and PSI, Oxford, MS -USA by LC-MS-MS
M. ElSohly and Waseem Gul

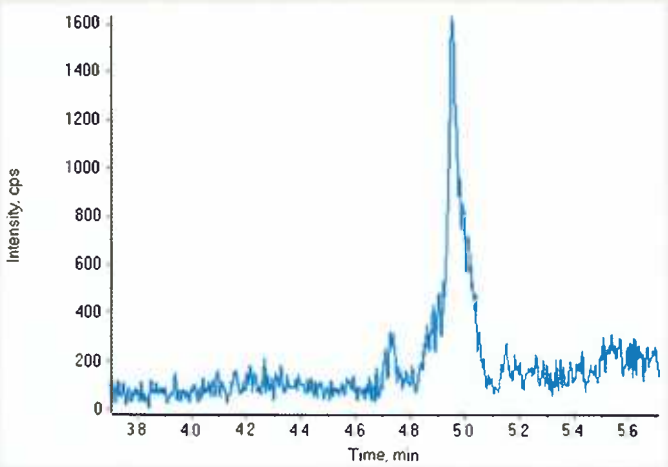
#.	Samples	Specimen	DMAA
1	Sample #1	*Pelargonium zonale* Aif, from Yunnan province, China	ND
2	Sample #2	*Pelargonium graveolens* L' Her, Yunnan province, China	ND
3	Sample #3	Provided by De' an Guo Yunnan province, China	ND
4	No 1: (little bottle)	Ni de lan Rose Geranium oil Provided by Fangli Biotechnology limited company Kunming, Yunnan province	ND
5	No 2: (little bottle)	Geranium oil	ND
6	No 3: (little bottle)	Geranium oil-2012	ND
7	No 4: (little bottle)	Geranium oil- no label	ND
8	1286 (Egypt)	NCNPR# 13151	ND
9	1653 (Gingind lovu)	NCNPR# 13152	ND
10	1758 (Ntsimbini)	NCNPR# 13153	ND
11	1759 (Nelspruit)	NCNPR# 13154	ND
12	1787 (Kristammahoek)	NCNPR# 13155	ND
13	*Pelargonium zonale* cv' daredevvk salmon' (stem)	NCNPR# 13039	
14	*Pelargonium zonale* cv' daredevvk salmon' (leaf)	NCNPR# 13040	ND
15	*Pelargonium graveolens* cv 'Bontrosai' (stem)	NCNPR# 13041	ND

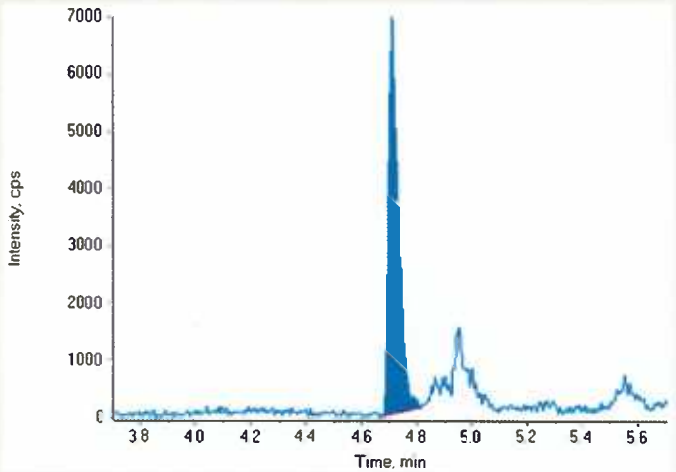
#	Samples	Specimen	DMAA
16	*Pelargonium graveolens* cv 'Bontrosai' (root)	NCNPR# 13042	ND
17	*Pelargonium graveolens* cv 'Bontrosai' (leaf)	NCNPR# 13043	ND
18	*Pelargonium graveolens* (leaf)	NCNPR #13044	ND
19	*Pelargonium graveolens* (stem)	NCNPR #13045	ND
20	*Pelargonium tomentosum* (leaf)	NCNPR# 13046	ND
21	*Pelargonium hortorum* (leaf)	NCNPR# 13047	ND
22	*Pelargonium hortorum* (stem/root)	NCNPR# 13048	ND
23	*Pelargonium hortorum* (flower)	NCNPR# 13049	ND
24	*Pelargonium odoratissimum* (whole plant)	NCNPR# 10591	ND
25	*Pelargonium tomentosum* (stem)	NCNPR# 10605	ND
26	*Pelargonium hortorum* (whole plant)	NCNPR# 10616	ND
27	*Pelargonium denticulatum* (leaf)	NCNPR# 10636	ND





Pelargonium Graveolens L' Her Yunnan Province
A: unspiked
B: Spiked with 10 ng/mL (3.9 ng/mL extracted)





Ni de lan Rose Geranium oil (Yunnan Province, China)
Provided by Fangli Biotech, (Kunming, Yunnan Province, China)

A: unspiked
B: Spiked with 10 ng/mL (6.26 ng/mL extracted)

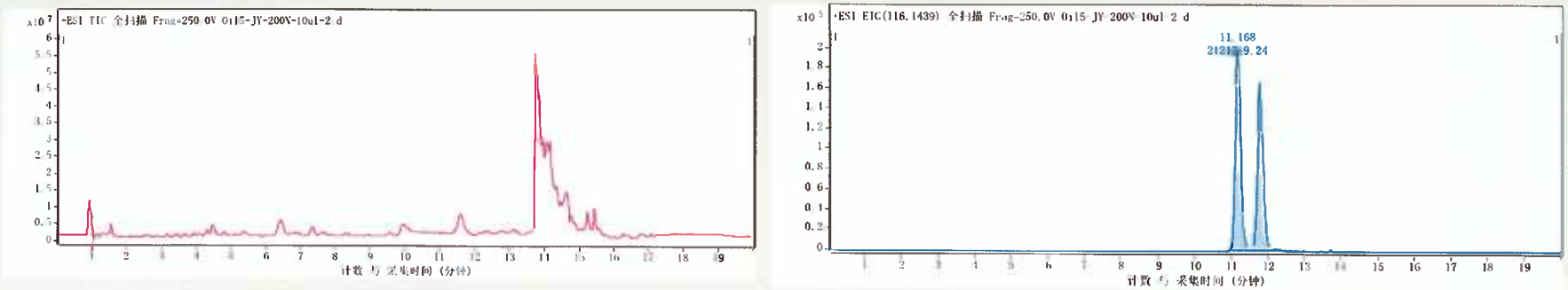
Wei-Dong Zhang and Juan Su
School of Pharmacy
Second Military Medical University Shanghai China

Apparatus and instruments

Agilent 1290 HPLC coupled with 6550 ifunnel Qtof was used. The analytical column is Agilent ZORBAX RRHD Eclipse Plus C18 column(3 mm×100 mm, 1.8 μm) (Agilent, CA, USA).

Sample			Result
Oil	NO.	place of production	
Oil-1	Egypt	Egypt	N.D.
Oil-2	Gingindlovu	Gingindlovu	N.D.
Oil-3	Ntsimbini	Ntsimbini	N.D.
Oil-4	Nelsruit	Nelsruit	N.D.
Oil-5	Kriskammahoek	Kriskammahoek	N.D.
Oil-6	No1	Kunming,China	N.D.
Oil-7	No2	Yunnan,China	N.D.
Oil-8	No3	Yunnan,China	N.D.
Oil-9	No4	Yunnan,China	N.D.
Plant		part	
Plant-1	pelargonium zonale	stem	N.D.
Plant-2	pelargonium zonale	leaf	N.D.
Plant-3	pelargonium graveolens	stem	N.D.
Plant-4	pelargonium graveolens	root	N.D.
Plant-5	pelargonium graveolens	leaf	N.D.
Plant-6	pelargonium graveolens	leaf	N.D.
Plant-7	pelargonium graveolens	stem	N.D.
Plant-8	pelargonium tomentosum	leaf	N.D.
Plant-9	pelargonium hortorum	leaf	N.D.
Plant-10	pelargonium hortorum	stem/root	N.D.
Plant-11	pelargonium hortorum	flower	N.D.
Plant-12	pelargonium odoratissimum	whole plant	N.D.
Plant-13	pelargonium tomentosum	stem	N.D.
Plant-14	pelargonium hortorum	whole plant	N.D.
Plant-15	pelargonium denticulatum	whole plant	N.D.
Plant-16	Pelargonium zonale Yunnan province China	whole plant	N.D.
Plant-17	Pelargonium graveolens Yunnan province China	whole plant	N.D.
Plant-18	Yunnan province China	whole plant	N.D.

N.D.: not detected (below LOD of 5 ng/mL or 28 ng/g)



A B

TIC and EIC of oil sample added with MHA standard solution(A: TIC; B: EIC)

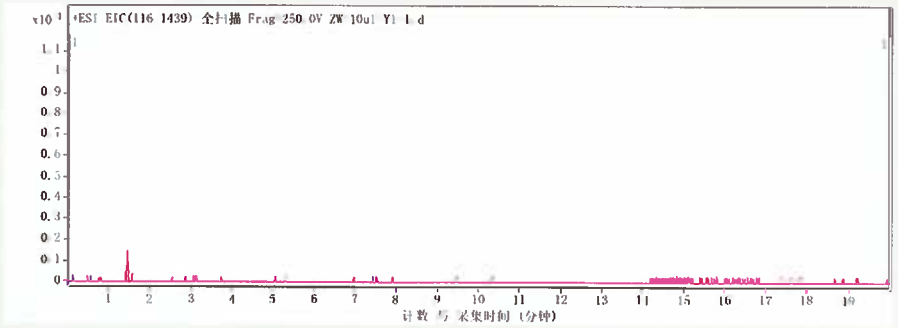


Figure 3 EIC of Plant-1

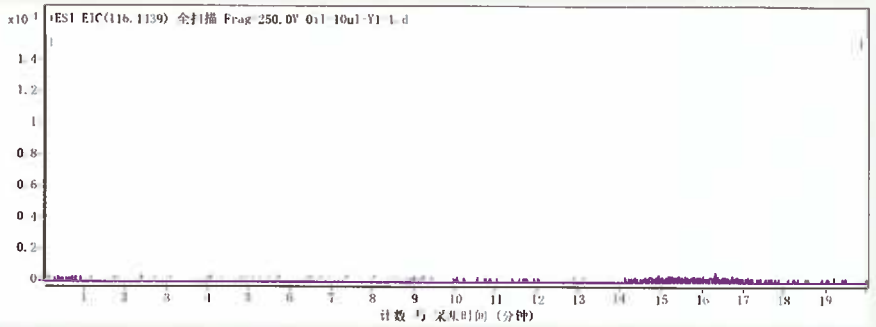


Figure 4 EIC of Oil-1

Conclusions

- A total of 27 samples of *pelargonium*, plant material (18) and *pelargonium* oil (9) collected from different parts of the world were used in this study.
- Four different institutions participated in the analysis of all 27 samples (split samples were provided to each institution).
- Extraction in all cases was performed according to the protocol published by Li *et al*.
- At the 10ppb (10ng/mL) detection level reported in our first study, none of the samples were found to be positive for DMAA.
- We conclude that, there is no question that DMAA found in commercial dietary supplements is of synthetic origion.

Acknowledgements:

The authors appreciate the efforts of all the participating institutions, those whose provided samples for analysis and those who actually performed the analysis for this study. Support for the initial study was provided by U.S antidoping Agency, Colorado Springs, CO, USA.

PSI
PHYTOCHEMICAL SERVICES INCORPORATED

eLLi

Exhibit 28

Despite checkered history, Norcross supplement maker avoids FDA crackdown

AJC investigation prompts retailers to remove products

ATLANTA-NEWS By Danny Robbins - The Atlanta Journal-Constitution

Hi-Tech Pharmaceuticals president and CEO Jared Wheat at the company's headquarters in Norcross, Georgia on Wednesday, December 5, 2007. The dietary supplement manufacturer is facing a variety of federal charges. PHOTO CREDIT: ERIK S. LESSER

Updated: 5:52 p.m. Saturday, November 02, 2013 | Posted: 12:00 a.m. Saturday, November 02, 2013

For more than a decade, federal authorities have had their eyes on Jared Wheat.

Wheat and his Norcross dietary supplement company, Hi-Tech Pharmaceuticals, have been forced to destroy products spiked with an erectile dysfunction drug, accused of selling a concoction containing a date-rape drug and and hit with a judgment of nearly $16 million for making false claims.

When federal prosecutors built a case against Wheat in 2009 for directing a scheme that sold knock-off prescription drugs through the Internet, they called him "a dangerous drug dealer." He went to prison for two years. He and his company were required to forfeit $3 million.

Now Wheat and Hi-Tech Pharmaceuticals have found another niche. In this case, his products have names like Yellow Scorpion, Black Widow, White Lightning and Lipodrene Hardcore. And, according to their labels, they contain an amphetamine-like stimulant that the U.S. Food and Drug Administration says is illegal.

The stimulant, dimethylamylamine, or DMAA, also has been linked to dozens of serious health problems, including heart attacks and strokes. At least eight people in the U.S. have died in the last three years after using DMAA products, according to lawsuits, military records and adverse event reports compiled by the FDA. Four were soldiers who used the products to get through physical training.

For more than a year, the FDA has been attempting to crack down on companies selling DMAA products, which are promoted largely as improving energy for workouts and aiding in weight loss.

Related

List of supplements with DMAA still on the market

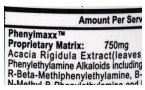
Adverse events associated with use of DMAA

Warning letter

Hi-Tech Pharmaceuticals website

Despite checkered history, Norcross supplement maker avoids FDA crackdown

Despite checkered history, Norcross supplement maker avoids FDA crackdown



Despite checkered history, Norcross supplement maker avoids FDA crackdown

But Wheat's companies, Hi-Tech Pharmaceuticals and a subsidiary, APS Nutrition, are among dozens that have continued to sell DMAA products in stores and online, The Atlanta Journal-Constitution found.

The AJC found a Hi-Tech Pharmaceuticals product labeled as containing DMAA on the shelves at GNC, as well as at CVS and Kroger stores. That supplement, Fastin-XR, is a weight-loss product that "seriously pumps up the stimulant activity" through DMAA and other ingredients, according to the Hi-Tech web site.

Inquiries from the AJC about the product caused both CVS and Kroger to recall it.

"We were not aware that Fastin-XR contained DMAA," said Keith Dailey, a spokesman for Kroger Co. "We're aware of it now."

GNC, a defendant in two wrongful death suits over DMAA, said it is no longer purchasing the products. But the Pittsburgh-based health and fitness giant would not say whether it would remove existing stock from its shelves.

In an interview with the AJC, the FDA's top supplement official, Dan Fabricant, acknowledged that the FDA was "familiar" with Wheat but wasn't aware his companies were selling DMAA products until informed by the newspaper.

None of the nearly 90 adverse event reports the agency has collected related to DMAA links Wheat's products to the medical problems. The majority cite supplements distributed by a Dallas company, USPlabs.

The FDA has sent letters to a handful of companies warning them that DMAA cannot be used in dietary supplements, and USPlabs agreed to destroy its stock of two popular products, Jack3d and OxyElite Pro, after the agency went to court to seize more than 3,200 cases in GNC warehouses.

Those actions aren't insignificant, Fabricant said.

"We've got a marketplace of 85,000 products out there," he said. "That's enormous. I think our actions against USPlabs, the biggest seller of this particular product … shows we don't take these matters lightly."

Some say the supplement industry can't be easily policed because, unlike drugs, supplements do not require FDA approval before they can be sold.

Still, the fact that Wheat's companies have avoided the FDA's crackdown raises new concerns about the agency's ability to purge an ever-burgeoning market of products that can cause harm, some industry experts said.

Dr. Pieter Cohen, an assistant professor at Harvard Medical School who has closely studied DMAA and its effects, said it's "just shocking" that, given Wheat's history, the FDA didn't immediately notice his companies were selling supplements with the stimulant.

Wheat, 41, declined a request to be interviewed for this story through his attorney, Art Leach.

"We certainly have a perspective on all that," Leach said, referring to the DMAA issue. "But this is not the best time to engage in that conversation."

Legal problems mount

Wheat started Hi-Tech Pharmaceuticals in the late 1990s, shortly after he completed a federal prison sentence for selling Ecstasy in his native Alabama.

Because he didn't finish college and had a felony conviction, Wheat believed he would be shut out of a more traditional business career, according to a bio that appeared on his company's web site.

"It was during a period of time I was (imprisoned) I basically formulated the idea of Hi-Tech and I came up with the name and started the company out of my parents' house in Birmingham," he was quoted as saying.

Wheat's business history has since played out against a backdrop of thousands of pages of court documents, some containing salacious allegations, as a variety of federal agencies have pursued him and his companies.

In 2003, Hi-Tech agreed to destroy seven of its products after the federal government sought an injunction against it for selling unapproved and misbranded drugs. Some of those products had been the subject of an earlier public health advisory stating they contained tadalafil, an erectile dysfunction drug that had yet to be approved in the U.S.

In the knock-off prescription drug case, the government portrayed Wheat as the mastermind of an enterprise that purported to sell cheap, generic drugs from Canada when in fact they were manufactured under unsanitary conditions in Belize.

Wheat was arrested at his Alpharetta home in 2006, and authorities seized two expensive sports cars _ a 2006 Maserati Quattroporte Q and a 2000 Ferrari 360 Modena F _ in addition to computers and more than $100,000 in cash.

Perhaps the most compelling part of the case were allegations put forth by the government in an effort to detain Wheat prior to trial.

Describing Wheat as “a dangerous drug dealer who has perhaps squirreled away millions of dollars and is a significant flight risk,” authorities quoted a witness who said he’d buried hundreds of thousands of dollars behind Wheat’s house at Wheat’s request. It also was alleged that Wheat and others had discussed hiring a private detective to blackmail an assistant U.S. attorney and obtaining a gun and silencer to attack an FDA agent.

In a 2008 interview with MSNBC, Wheat denied having knowledge of the alleged plots.

“I’ve never been in as much as a fistfight since I was a 10-year-old kid,” he said. “I’m not a violent person and Hi-Tech is not that kind of company.”

Wheat ultimately pleaded guilty to conspiracy to commit mail and wire fraud and selling unapproved and adulterated drugs and was sentenced to 50 months in prison. He remains on supervised release as part of his sentence.

Four of Wheat’s co-conspirators also received prison terms.

“We are extremely fortunate that no one was sickened or killed by these drugs,” said David Nahmias, then the U.S. attorney for the northern district of Georgia.

Wheat and his companies have also been embroiled in a lawsuit filed by the Federal Trade Commission seeking sanctions against them for making false and unsubstantiated product claims.

The lawsuit, filed in 2004, led to a 2008 judgment requiring that Wheat and other defendants pay $15.9 million and refrain from making certain product claims. But the case remains unresolved, with Wheat facing the possibility of jail time for contempt.

The FTC alleges that Wheat and his companies have continued to make false claims, causing consumers to lose at least $36 million. “*Only* coercive incarceration” will compel them to comply with the court’s order, the FTC said in a September court filing.

U.S. District Judge Charles A. Pannell will consider the matter at a hearing that has yet to be scheduled, according to court documents.

Among the documents filed by the FTC are emails indicating that, even while incarcerated, Wheat directed Hi-Tech, often with an eye to making sure the company’s products would be sold by GNC and other national retail outlets.

“The only way to pay down the FTC debt is to hit a home run,” Wheat wrote in an email to his associates. “I have done well over the years with base hits but now I have to swing for the fence.”

‘Jolt of extreme energy’

According to the Washington Post, a chemist, Patrick Arnold, introduced DMAA as a supplement ingredient in 2006, calling it “Geranamine.” Arnold had previously gained notoriety for his role developing substances for the Bay Area Laboratory Cooperative, or BALCO, the company famously exposed as a supplier of performance-enhancing drugs for athletes.

Hi-Tech Pharmaceuticals introduced its first DMAA products about the time Wheat was released from prison in March 2011 and added more in 2012, an Internet Archive search of the company’s website shows.

Around the same time, a series of deaths linked to USPlabs’ DMAA products started to call attention to the stimulant.

Michael Sparling collapsed during a run at Fort Bliss in El Paso, Texas, in June 2011. Before the exercise, the 22-year-old Army private from California used the USPlabs product Jack3d purchased at a GNC store on the base, according to a wrongful death suit filed by his family.

The death of a 24-year-old Abilene, Texas, woman while visiting friends in Austin in November 2011 occurred under similar circumstances, according to a lawsuit filed by her family. Jessica Davila died of malignant hyperthermia after using USPlabs' OxyElite Pro, also purchased at GNC, the suit says.

Mark Zamora, an Atlanta attorney who is representing Davila's estate, called Davila "a fit young lady who took her health seriously."

"She took the typical dosage and paid for it with her life," he said.

The deaths of Sparling and another soldier prompted the Department of Defense to remove all DMAA products from military exchanges.

The FDA then got involved. It says DMAA is illegal because, even though some products label it "geranium extract," it is a synthetic compound. That means it doesn't qualify under federal regulations for use in dietary supplements because it is not a natural ingredient.

The FDA began sending out warning letters to companies selling DMAA in April 2012 and has since sent a total of 13. It's believed all have stopped selling the products, Fabricant said. Two other companies have voluntarily recalled their DMAA products.

However, a database of DMAA products maintained and updated monthly by the Human Performance Research Center, a federal program that studies health issues affecting soldiers, shows that 69 products distributed by 40 different companies remain available online.

Eight of those products are from Hi-Tech Pharmaceuticals or APS Nutrition. On the companies' websites, DMAA is plainly listed among the ingredients _ in some cases, boldly so.

"Millions of people have enjoyed the effects of … DMAA," the description for one of the products proclaims. "… Now White Lightning takes extreme energy to another level! White Lightning will cause you to feel the jolt of extreme energy from the moment you take it and will stay all over you for hours!"

Spot checks by the AJC at retail outlets in two states also found one of Hi-Tech's DMAA products, Fastin-XR, on the shelves at GNC, CVS and Kroger stores.

Mike DeAngelis, a spokesman for CVS Caremark, wrote in an email that, because no FDA warning letter had been sent to Hi-Tech Pharmaceuticals, CVS did not "have visibility to the DMAA issue" as it relates to Fastin-XR until contacted by the AJC.

Like Kroger, CVS ordered the product removed from its shelves.

GNC took a different stance when contacted by the newspaper.

In an email, company spokeswoman Laura Brophy said GNC "believes DMAA is safe and legal." She cited a Department of Defense report that found no "direct causality" between the military deaths and DMAA.

Although Brophy said GNC is no longer purchasing products containing the stimulant, she said the company would not respond to the question of whether it would remove any existing products with DMAA from its shelves.

GNC CEO Joseph Fortunato, questioned about DMAA during the company's first quarter earnings call in April, said the FDA had "pretty much" said the products are safe "just from the fact they haven't pulled them from the shelves."

Fabricant defended the FDA's actions, saying the agency is doing its best with limited resources.

"One out of every four dollars in the country is spent on an FDA-regulated good," he said. "It's pretty clear we don't command one out of every four dollars in the (federal) budget, nor is one out of every four people in the U.S. working for the FDA."

Steve Mister, president of the Council for Responsible Nutrition, a trade group for supplement companies, said the FDA typically cracks down on market leaders and hopes smaller firms will comply on their own.

“They hope the little guys see that the big guy is facing a seizure and the destruction of product,” he said. “But if (the smaller firms) don’t get the message, then the FDA does need to go back and clean up the smaller players as well.”

The FDA has had the authority to issue mandatory recalls for supplements since Congress passed the Food Safety Modernization Act in 2010, but the agency has yet to use it.

Zamora said the FDA’s effort is failing, and it won’t become effective unless there’s a mandatory recall of all DMAA products.

“If there’s another death, it’s on the FDA and the product maker,” he said.

1944 _ Dimethylamylamine, or DMAA, is patented under the name Forthane by Eli Lilly and Company for use in nasal spray.

1983 _ Eli Lilly withdraws Forthane from the market.

2006 _ Patrick Arnold, a chemist involved in the BALCO steroid scandal, introduces DMAA as a dietary supplement ingredient under the name Geranamine.

June 2011 _ Army Pvt. Michael Sparling, 22, collapses after a run at Fort Bliss in El Paso, Texas, and dies. Hypothermia is listed as the cause of death. Toxicology tests show DMAA and caffeine in his blood.

November 2011 _ A 31-year-old female soldier collapses while running. She dies six weeks later. The cause of her death is also listed as hypothermia, and toxicology tests also show caffeine and DMAA.

December 2011 _ The Department of Defense orders that all products containing DMAA be pulled from military exchanges.

April 2012 _ The FDA begins sending warning letters to companies selling products with DMAA, advising them that those products can’t legally be sold as dietary supplements.

January 2013 _ A coroner in Britain cites DMAA as a factor in the death of 30-year-old runner Claire Squires at the 2012 London Marathon.

February 2013 _ Sparling’s family files suit against USPlabs, the Dallas company that distributed the dietary supplement Jack3d, and GNC, the retailer that sold it, alleging they contributed to the Army private’s death.

April 2013 _ The FDA issues an alert to consumers stating that DMAA doesn’t qualify as a legal supplement and that it can dangerously elevate blood pressure.

May 2013 _ The House Energy and Commerce committee requests information on DMAA from the FDA, USPlabs and GNC.

June 2013 _ The Department of Defense releases a report on DMAA. It says no “direct causality” could be found between the deaths of four soldiers and their use of DMAA products. However, it recommends continuing the ban on the sale of DMAA products at military exchanges.

June-July 2013 _ In federal court, the FDA seeks to seize more than 3,200 cases of USPlabs DMAA products Jack3d and OxyElite Pro at GNC warehouses in Pennsylvania and South Carolina. GNC subsequently agrees to destroy the products.

July 2013 _ USPlabs destroys its inventory of Jack3d and OxyElite Pro.

1998 _ Hi-Tech Pharmaceuticals is incorporated in Georgia.

2000 _ The Atlanta field office of the Drug Enforcement Administration investigates Wheat's role in the distribution of a substance called "Verve," which allegedly includes the date-rape drug Gamma Hydroxybutyrate (GHB) and is labeled as "cleaning solution" to conceal its real purpose. Wheat wasn't charged with a crime.

2003 _ Hi-Tech Pharmaceuticals agrees to destroy seven of its dietary supplements after the FDA seeks an injunction against the company for selling misbranded and unapproved new drugs. Some of the products had been subject to a public health advisory stating that they contained the erectile dysfunction drug tadalafil.

2004 _ The Federal Trade Commission files suit against Wheat, Hi-Tech Pharmaceuticals and others alleging that they made false advertising claims.

February 2006 _ The FDA seizes more than 200 cases of supplements valued at $3 million from Hi-Tech Pharmaceuticals because they contain the banned stimulant ephedra, linked to serious illnesses and deaths.

September 2006 _ A federal indictment alleges that Wheat masterminded a scheme in which Hi-Tech Pharmaceuticals used the Internet to sell knock-off prescription manufactured in Belize.

August 2008 _ Wheat pleads guilty to conspiracy to commit mail and wire fraud and to introduce and deliver unapproved new and unadulterated drugs into interstate commerce.

December 2008 _ U.S. District Judge Charles A. Pannell orders Wheat and other defendants in the FTC case to pay $15.9 million. The ruling also enjoins them from making false product claims in the future.

February 2009 _ Wheat is sentenced to 50 months in prison, followed by three years of supervised released.

March 2011 _ Wheat is released from prison.

August 2013 _ Pannell rules that Wheat and other defendants in the FTC case violated the 2008 court order by continuing to make false product claims.

September 2013 _ In a court filing, the FTC says it believes "coercive incarceration" is the only way to compel compliance with the judge's order.

About this story

To check on the availability of products labeled as containing dimethylamylamine, or DMAA, The Atlanta Journal-Constitution purchased products from the websites of both Hi-Tech Pharmaceuticals and APS Nutrition. The newspaper also found a Hi-Tech product with DMAA, Fastin XR, being sold at GNC, CVS and Kroger stores in the Atlanta metro and the Dallas-Fort Worth areas.

Material on the deaths and health issues relating to DMAA and the FDA's response was gathered from FDA documents, lawsuits and a report compiled by the Department of Defense, as well as interviews with industry experts. The AJC used the federal Freedom of Information Act to obtain a compilation of the adverse health events reported to the FDA.

To gain an understanding of how many supplement companies are still selling DMAA products, the AJC reviewed a database of DMAA products maintained by a federal program that studies health issues affecting soldiers.

Background on Jared Wheat was gleaned from criminal and civil court records dating back 10 years.

About the Author

DANNY ROBBINS

Sign up for Newsletters

Want more news? Sign up for free newsletters to get more of the AJC delivered to your inbox.

Reader Comments 0

Next Up in Local

Atlanta City Council OK's property damage resolutions

YEAR IN REVIEW: 5 stories that made Gwinnett smile in 2016

Senate panel: Consider state funding for mass transit by 2018

Clayton County approves grave relocation

Housing prices could help explain Hillary Clinton's victories in Cobb, Gwinnett

More Stories

Popular in News

1 Convicted kingpin left trail of blighted houses on Atlanta's westside

2 The foundation of democratic trust is crumbling | Jay Bookman

3 Georgia GOP Senate candidate says bankruptcy gives him economic insight | Political Insider

NEWS ▸

CUSTOMER SERVICE ▸

MARKETPLACE ▸

SOCIAL MEDIA ▸

Exhibit 29

Lot# 256-2257
Exp. Date 0716

SUPPLEMENT FACTS

Serving Size: 1 Tablet Servings Per Pack: 2	Amount Per Tablet	% Daily Value*
Proprietary Blend: with Thermo-RX® and Extend-Rx™ Technology	**510mg**	**
25mg Ephedra Extract (leaves), Acacia Rigidula Extract (leaves) [Yielding 125mg Phenylethylamine Alkaloids: Methylsynephrine, R-Beta-Methylphenylethylamine, B-Phenylethylamine,N-Methyl-B-Phenylethylamine], Theobroma Cocoa Extract (seed), Phenylethylamine HCl, Citrus Aurantium Extract (25mg Synephrine), Green Tea Extract (leaves), 1,3 Dimethylamine HCl,Yohimbe Extract (bark), Naringen (fruit), 6, 7 Dihydroxybergamottin		
Caffeine (anhydrous)	**150mg**	**

* Based on a 2000 calorie diet. ** Daily value not established

Other Ingredients: Microcrystalline Cellulose, Dextrose, Sodium Starch Glycolate, Magnesium Stearate, Stearic Acid, Silica, FD&C Blue #1, FD&C Red #40

Dosage: Take 1 tablet three times daily. Do Not exceed 4 tablets in any 24 hour period.

Manufactured for: Hi-Tech Pharmaceuticals, Inc.
6015-B Unity Drive • Norcross, GA 30071 • 1-888-855-7919

Warning: Do not use by individuals under the age of 18 years. Do not use if you are pregnant or nursing. Consult a physician or licensed qualified health care professional before using this product if you have, or have a family history of, heart disease, thyroid disease, diabetes, high blood pressure, recurrent headaches, depression or other psychiatric condition, glaucoma, difficulty in urinating, prostate enlargement, or seizure disorder, or if you are using a monoamine oxidase inhibitor (MAOI) or any other dietary supplement, prescription drug or over-the-counter drug containing ephedrine, pseudoephedrine, or phenylpropanalamine (ingredients found in certain allergy, asthma, cough/cold, and weight control products). Do not exceed recommended serving. Exceeding recommended serving may cause serious adverse health effects, including heart attack and stroke. Discontinue use and call a physician or licensed qualified health care professional immediately if you are experiencing rapid heartbeat, dizziness, severe headache, shortness of breath, or other similar symptoms. Individuals who consume caffeine with this product may experience serious adverse health effects. Individuals who are sensitive to the effects of caffeine should consult a licensed health care professional before consuming this product. In case of overdose, seek professional assistance or contact a poison control center immediately. Avoid alcohol while taking this product.

Do Not Use If Outer Seal Is Broken Or Missing.

8 57084 00076 7

Exhibit 30

Hi-Tech Pharmaceuticals, Inc.

PRODUCT: Black Widow 4ct

AMOUNT: 24-24 Packs

LOT: 10097

EXP: 10/2015