Short communication

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Could 1,3 dimethylamylamine (DMAA) in food supplements have a natural origin?

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1,3 dimethylamylamine or methylexaneamine (DMAA) is a synthetic pharmaceutical patented in the 1940s as a nasal decongestant which can be used as a recreational stimulant. Alleged to occur in nature, DMAA has become a widely used ingredient in sports food supplements, despite its status as a doping agent and concerns over its safety. There is now some doubt as to whether it can be sourced naturally or whether it actually occurs naturally at all. The presence of DMAA was investigated by high performance liquid chromatography (HPLC) in extracts of the leaves and stems of four geranium species and of three well-known cultivars. The amounts of DMAA in commercial geranium (*Pelargonium graveolens*) oil and the leading sports supplement which uses the ingredient were also measured. DMAA was not found in any of the leaves or stems or in the commercial geranium oil included in this study. Approximately 30 mg per daily dose was found in the food supplement. Therefore, the amount of DMAA found in the supplement is most unlikely to have been sourced in nature, and it must be concluded that synthetic DMAA, known to be capable of causing severe adverse physiological effects, has been added.

Keywords: 1,3-dimethylamylamine; DMAA; doping; *pelargonium*

Introduction

Many pharmaceutical compounds were originally identified in plants. In 1996, Ping *et al*.[1] reported that methylhexaneamine, a pharmaceutical compound originally discovered and produced synthetically,[2] is a natural component of the steam-distilled volatile oil obtained from the leaves and stems of *Pelargonium graveolens* L'Hér.

Methylhexaneamine or 1,3-dimethylamylamine (DMAA), an aliphatic amine,[3] was originally patented in 1944 by Eli Lilly as a nasal decongestant. Like ephedrine and amphetamines,[1] it has vasopressor activity and displays sympathomimetic properties.[4–6] A food supplement called Jack 3D containing 1,3-dimethylamylamine was recalled by Health Canada as an unapproved drug under the Food and Drug Regulations.[7]

To date, Ping *et al.'s* study[1] is the only one to have found the compound in geranium oil, but since 2006 at least, food supplement manufacturers have included DMAA and labelled it as geranium oil, geranium stem or, in the EU, as *Pelargonium graveolens* leaves dry extract, stating it to be of natural origin.[8] The substance is also used in New Zealand[9] and Ireland[10] as a 'party-pill'. Several adverse events (headache, vomiting, nausea, hypertension, cerebral haemorrhage), consistent with the known properties of DMAA, have been reported in New Zealand.[9,11] DMAA is listed as a stimulant doping substance by the World Anti-doping Agency (WADA).[12] Perrenoud *et al*.,[13] in testing for doping, reported the presence of 350 ng/ml of DMAA in urine up to four days after the intake of 40 mg of the amine contained in a food supplement, Geranamine (Predator Nutrition, UK).

More recently, severe adverse events (cardiotoxicity, liver and kidney damage, death) have been reported in the USA.[14,15] However, products with DMAA remain on sale there (despite, recently, FDA-issued warning letters to ten manufacturers and distributors of dietary supplements containing dimethylamylamine for lack of safety evidence[16]) and, in practice, in most of the EU also, thanks to the Internet market.

One of the key questions has been whether DMAA can actually be obtained from geranium extracts used as an ingredient in food supplements. Health Canada has determined, after reviewing a number of studies, that DMAA cannot be sourced from geranium, and has forbidden its use in food supplements (more precisely, under Canadian law, in Natural Health Products).[7] A similar outcome would be likely in the EU, where extracts of geranium (which are used in flavourings) are generally permitted for use in food supplements, whereas synthetic DMAA would not be. In the present study, we have investigated whether leaves and stems of *Pelargonium graveolens* L'Hér, of three of its cultivars and of three related species contain DMAA. We also measured the content of DMAA in commercial geranium oil and in the relative leading sport food supplement.

Materials and methods

Plant samples

The species/cultivars of *Pelargonium* (*Geraniaceae*) included in this study were botanically certified and provided by the plant nursery *Il peccato vegetale* (Usmate-Velate, MB, Italy) on 3 November 2011. They were: *Pelargonium citriodorum* Hort. ex Breiter, *Pelargonium denticolatum* Kuntze, *Pelargonium graveolens* L'Hér, *Pelargonium*

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tomentosum Jacq, *Pelargonium* 'Creamy Nutmeg', *Pelargonium* 'Sarah Jane', *Pelargonium* 'Sweet mimosa'.

Leaves and stems of each plant were removed, stored at −20 °C under vacuum.

Geranium oil

Commercial geranium oil *Note di cuore* – geranium Africa, containing an extract from *Pelargonium graveolens* leaves, was supplied by Erboristeria Magentina, Poerino, TO, Italy.

Dietary supplement

The dietary supplement analyzed was Jack 3D, from USPlabs, LLC (Dallas, TX, USA).

According to the label, the product contained as active ingredients arginine alpha-ketoglutarate, creatine monohydrate, beta-alanine (CarnoSyn®), caffeine, 1.3-dimethylamylamine (geranium, stem), schizandrol A (*Schisandra chinensis*, berry), along with citric acid, natural flavours, acesulfame potassium, sucralose, silicon dioxide, vegetable stearate, beta-carotene (for colour). No information about DMAA content was reported. The suggested dose was 5.55 g of powder up to three times daily. Each container provided 45 servings.

Table 1. Data on recovery obtained by six samples at two different spiking values

Sample	Added (μg/g)	Calculated (μg/g) (mean ± SD)	Recovery (%) (mean ± SD)
Stems	4	3.94 ± 0.12	98.5 ± 3.0
	15	14.66 ± 0.75	97.7 ± 5.0
Leaves	4	3.89 ± 0.15	97.1 ± 3.8
	15	14.69 ± 0.54	97.9 ± 3.6

Chemicals

All solvents and reagents had analytical-grade purity. Methanol (99.8 % purity), high performance liquid chromatography (HPLC) grade water, anhydrous sodium acetate (≥ 99% purity) and phthaldialdehyde reagent (OPA, ≥ 99% purity) were obtained from Sigma-Aldrich Chemie, (Schnnelldorff, Germany); HPLC-grade tetrahydrofuran (≥ 99% purity) was from Merck (Darmstadt, Germany). Reference compound 1,3-dimethylamylamine (DMAA) was from Sigma-Aldrich (Sigma-Aldrich Library of Rare Chemicals, Milwaukee, WI, USA).

Apparatus

The HPLC analysis was performed using a a Spectra System P2000 gradient pump, a Spectra System UV6000 LP Diode Array Detector (DAD), each one purchased from Thermo Separation Products (San José, CA, USA). The data were collected by ChromQuest Software (San José, CA, USA).

The column was a 4.6 x 250 mm, Synergi, 4 μm, MAX-RP, 80 A (Phenomenex, Torrance, CA, USA) maintained at 45 °C in a model 7971 column thermostat (Jones Chromatography, Hengoed, Mid, Glamorgan, UK). For some samples a LichroCart 250–4 mm, RP18 5 μm, Lichrospher 100 (Merck, Darmstadt, Germany) column was used at 45 °C.

Standard preparation

The standard compound, 10 mg, was dissolved in 10 ml of methanol and stored at −20 °C. Appropriate dilutions of this stock solution were prepared and stored at 4 °C until use. From each solution, three aliquots of 0.2 ml were added with 0.2 ml OPA. The obtained derivatives, stored at room temperature in the dark, were injected in HPLC column within 15 min. Standard solutions were analyzed in the range 1.56–12.5 μg/ml.

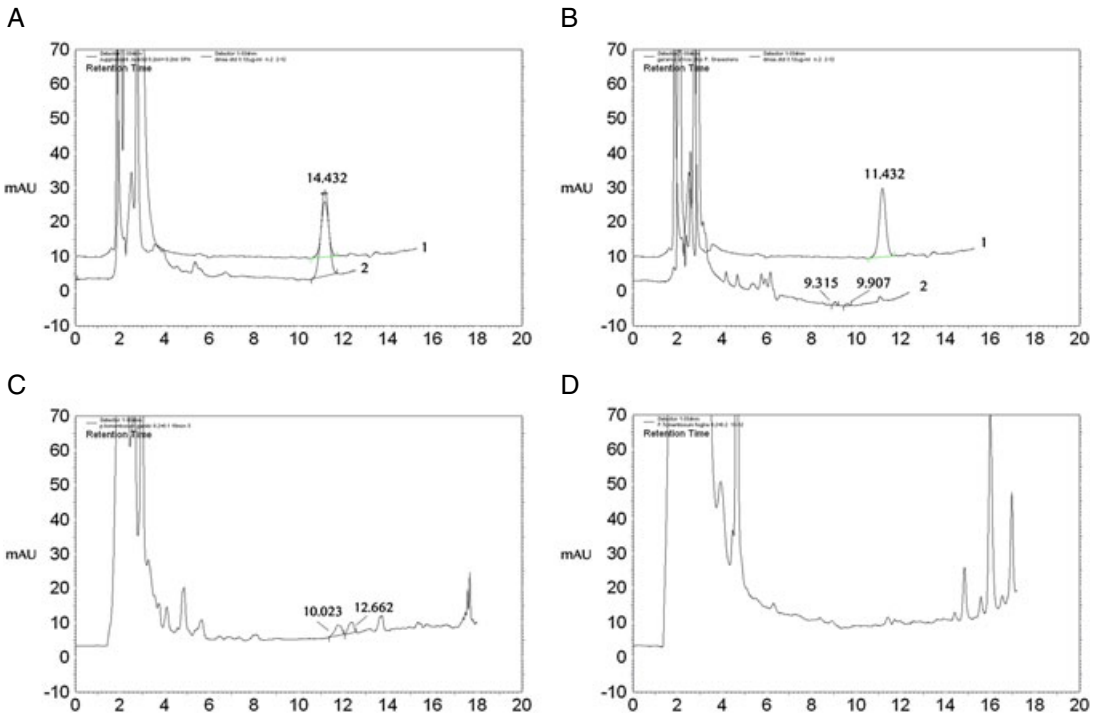

Figure 1. HPLC chromatograms at 334 nm of: (A) Jack 3D food supplement (line 2); (B) commercial *P. graveolens* oil (line 2); (C) methanolic extracts of stems (left) and leaves (right) of *P. tomentosum*. In (A) and (B) line 1 represents the standard solution containing 3.12 μg/ml DMAA.

Sample preparation

Plants

Appropriate amounts of fresh stems and leaves were finely chopped and extracted with methanol under reflux for 3 h. Samples were suspended at the final concentration of 100 mg/ml apart from *P. citriodorum* prepared at 40 mg/ml. The methanol extracts were filtered through filter paper and evaporated to dryness. The residue was suspended in 1–2 ml of methanol, and aliquots (0.2 ml) of this solution were derivatized following the procedure described for standard solutions.

Geranium oil

A volume of 200 μl of oil was diluted with 2 ml of methanol and filtered. Aliquots of 0.2 ml were treated as above.

Dietary supplement

The powder was quartered and mixed before sampling. Exactly 1 g of the dietary supplement was easily solubilized in 200 ml methanol. Aliquots of 0.2 ml were treated as described above and filtered just before HPLC injection.

HPLC analysis

DMAA was measured as its o-phthalaldehyde (OPA) derivative using a validated HPLC-DAD (Diode Array Detector) method. Samples were injected into a chromatographic reverse-phase column with linear gradient elution using as mobile phases a mixture of: 0.05 M sodium acetate buffer:tetrahydrofuran (96:4 *v/v*) (A) and 100% methanol (B). The percentage of B was 70% for the first 7.5 min, then increased to 100% at 15 min, and remained isocratically for 10 min.

The column was connected to a DAD for quantitative analysis. Standard and test samples were monitored between 254 and 500 nm and measured at 334 nm, corresponding to the maximum absorbance of amine-OPA derivatives.

Assay validation

Linearity

The linearity was assessed in the concentration range 1.56–12.5 μg/ml of the final solution of the derivatized amine. Five points of different concentrations were obtained by diluting the stock solution. For each point the injection was repeated three times. The regression

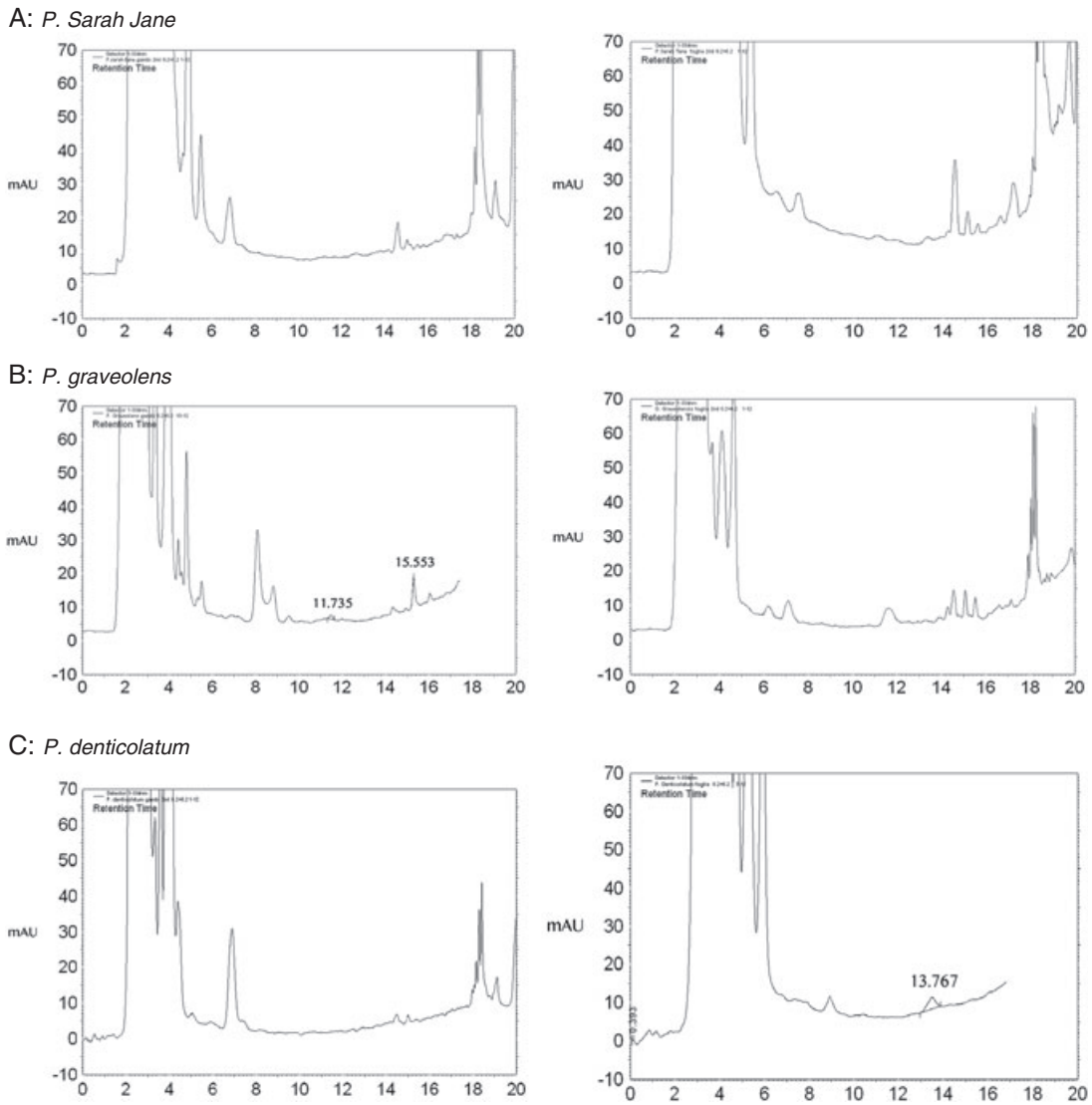

Figure 2. HPLC chromatograms at 334 nm of methanolic extracts of stems (left) and leaves (right) of (A) P. *Sarah Jane*, (B) *P. graveolens*, (C) *P. denticolatum*.

coefficient (R^2) and the parameters of the calibration curve were calculated.

Precision

The intra-day and the inter-day precision of the method were evaluated by injecting six times a standard sample on the same day and in three different days. The values of relative standard deviations (SD) for peak area and retention times were calculated.

Specificity

The reagent and solvents used in the method were submitted to the whole extraction procedure applied for the samples.

Recovery

Recovery was calculated by adding two known amounts of DMAA (40 μg and 150 μg in 1 ml methanol) to 10 g of stems and leaves just before the extraction procedure. This procedure was repeated three times. The percentage recovery was obtained by comparing the area of these samples with the area of the authentic standard (%) and calculating mean ± SD.

Results and discussion

The HPLC/DAD method developed for this research showed a suitable sensitivity and specificity for the determination of minute amounts of DMAA in plants, oil and food supplements.

The reaction with OPA develops with high reproducibility, at room temperatures and in a few minutes, stable derivatives having high UV absorption. The sensitivity and specificity of the method are comparable with the method described by Lisi *et al.*[17] where gas chromatogprahy-mass spectrometry (GC-MS) was used.

Assay validation

A good linearity was observed with a R^2 0.9906 ± 0.0015 in the range analyzed (1.56–12.5 μg/ml). The values of slope and intercept were calculated by six independent calibration curves and were 140154 ± 4807 (RSD% 3.43) and 77285 ± 10124 (RSD% 13.1), respectively.

Intra-day data of the method were: for retention times 12.45 ± 0.33 (RSD% 2.65) and for area, associated with the standard concentration 10 μg/ml, 2416638 ± 44950 (RSD% 1.86). Similarly,

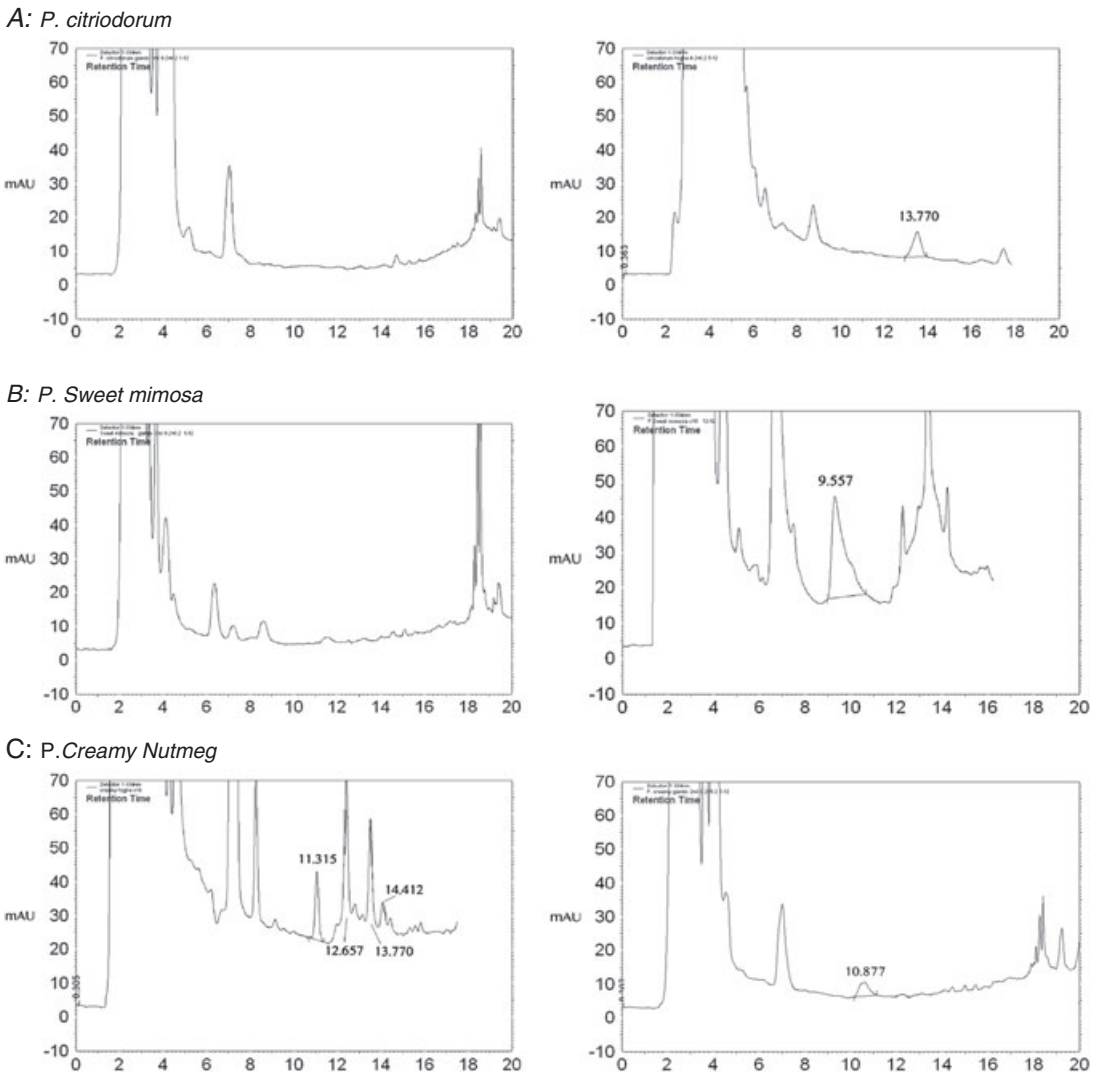

Figure 3. HPLC chromatograms at 334 nm of methanolic extracts of stems (left) and leaves (right) of (A) *P. citriodorum*, (B) *P. sweet mimosa* and (C) *P. Creamy Nutmeg*.

inter-day precision results were 12.17 ± 0.46 (RSD% 3.77) and 2488314 ± 97542 (RSD% 3.92).

No peak at the retention time corresponding to DMAA was present in the blank extract, showing that the extraction solution contains no compound interfering with the analyte.

The limit of detection (LOD) of 0.300 μg/ml and the limit of quantitation (LOQ) of 1.56 μg/ml were determined as a signal-to-noise-ratio (S/N) 3:1 and 10:1, respectively. These values corresponded to 0.6 μg/g stems and 1.2 μg/g leaves.

The recovery of DMAA at low concentration (4 μg/g) from stems and leaves was 98.50 ± 2.96 (RSD% 3.00) (Table 1). Similarly, at high concentration (15 μg/g), the DMAA recovery was 97.70 ± 4.88 (RSD% 5.00).

Sample quantification

Food supplement

Figure 1 (panel A) shows the chromatograms of the food supplement (line 2) and DMAA standard solution (3.12 μg/ml, line 1). The DMAA concentration from four independent preparations was 1.66 ± 0.30 mg/g (mean ± SD). The coefficient of variation (CV) was 18.4%, due to the heterogeneity of the food supplement composition, which could not be entirely resolved by mixing and quartering. The level of DMAA measured in the Jack 3D food supplement corresponded to a maximum daily dose (5.55 g x 3 times) of 27.6 ± 1.22 mg.

Pelargonium samples and geranium oil

One of the most discussed points was whether DMAA contained in sport food supplements could be of natural origin. This aspect does not change the possible risk for human health but could change the responsibility of producers. For this reason, stems and leaves from several Pelargonium varieties were analyzed to confirm or exclude the natural presence of DMAA. According to Lisi *et al*., no detectable amount of DMAA was found in geranium oil.[17] The natural presence of DMAA in geranium oil was described only by Ping *et al*.,[1] but the identification described in this publication was made by MS spectrum only, using the library and without comparison with authentic DMAA standard. Other authors[17] defined the results by Ping *et al*.[1] inconsistent and based on erroneous assignment of MS spectrum.

No detectable quantity of DMAA was found in the sample of commercial geranium oil analyzed in this research (Figure 1, Panel B).

Figure 1 (Panels C and D) shows the chromatograms obtained by injecting the extracts of stems and leaves of *P. tomentosum*. The chromatogram of stem shows a peak close to 12 min, but the presence of DMAA was excluded by co-injection (RT 12.66 versus 12.16, data not shown) and by comparing the UV spectrum of its derivative with that of OPA-DMAA one.

The chromatograms of the further extracts, coming from stems and leaves of six Pelargonium species/varieties (Figures 2 and 3), were very similar and no detectable amount of DMAA was found.

Our results indicate that DMAA is either (1) absent from geranium, (2) present at very low concentrations (below 1.2 μg/g), (3) occurs under rare and not yet described growing conditions, or (4) is present in very uncommon cultivars.

Our results on four species of *Pelargonium* and three known cultivars (including that reported on the label of Jack 3D), confirm experimentally the conclusion by other authors that DMAA cannot be sourced naturally.[3,7,17–19]

Conclusions

Salinger *et al*.[14] reported a case of a variant of Takotsubo cardiomyopathy, confirmed by echocardiography and magnetic resonance imaging (MRI) in a patient who worked for the distributor of Jack 3D®. This patient reportedly increased the DMAA concentration of Jack 3D 1.5 times, for personal use, to achieve a more 'energized' state.

Gee *et al*.[9] reported the case of a 21-year-old male who suffered a cerebral haemorrhage shortly after ingesting two capsules ('BZP-free' party pills), containing 278 mg/capsule of DMAA.

No detectable amounts of DMAA were found in the geranium species analyzed and its presence in sport supplements indicates that synthetic amine has probably been added.

Since DMAA is a sympaticomimetic agent, its presence could determine severe adverse effects in people using these food supplements in sport or in recreational activities. As a consequence, the addition of DMAA to food supplements must be considered a doping action aimed to improve athletes' performances. The absence of DMAA in other geranium products has been recently shown by ElSohly *et al*.[19]

The development of the validated analytical method described in this paper allows the control and quantification of DMAA, in order to protect consumers for the adverse effects associated with unsafe food supplements.

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