Short communication

Drug Testing and Analysis

Received: 5 August 2011 Revised: 4 November 2011 Accepted: 4 November 2011 Published online in Wiley Online Library: 6 December 2011

(wileyonlinelibrary.com) DOI 10.1002/dta.392

Studies of methylhexaneamine in supplements and geranium oil

A. Lisi,* N. Hasick, R. Kazlauskas and C. Goebel

A number of supplements are now available which are sold as fat burners or pre-workout boosters and contain stimulants which are banned in sport. Many contain methylhexaneamine under one of many pseudonyms including Geranamine, geranium oil or extract, or a number of chemical names such as 1,3-dimethylpentylamine. This has resulted in many athletes returning an adverse finding and having sanctions imposed. Other stimulants such as caffeine, phenpromethamine, synefrine, and phenethylamines are also to be found in supplements.

This communication shows that geranium oils do not contain methylhexaneamine and that products labelled as containing geranium oil but which contain methylhexaneamine can only arise from the addition of synthetic material.

Since the usual dose of methylhexaneamine is large, the drug is excreted at relatively high amounts for more than 29 h, the time for which the excretion was studied.

Keywords: Geranamine; Stimulant; supplements; methylhexaneamine; geranium oil

Introduction

A number of supplements which are advertised for weight loss contain compounds which can be classified as stimulants or are related to known stimulants. Stimulants have been used as anorectic substances to aid in appetite suppression by the pharmaceutical industry for some time with compounds such as phentermine, sibutramine, benfluorex, diethylpropion, amphetamine, benzphetamine, and phenethylamine being available and included on the World Anti-Doping Agency (WADA) Prohibited List.[1,2] Other stimulants such as benzylpiperazine, phenpromethamine, synefrine, and phenylethylamines have also been sold as 'supplements' or components of supplements and have caused athletes to return an adverse analytical finding with subsequent sanctions. This seems to be a trend that has recently surfaced where weak stimulants are added to supplements to allow them to be advertised as slimming agents. Recently the use of 2-amino-4-methylhexane (1,3-dimethylpentylamine, methylhexaneamine) has become widespread in such supplements resulting in a plethora of adverse findings since its detection has been published and implemented by anti-doping laboratories.[3–5] Invariably the athletes have stated that they were unaware of the material's presence in the preparation and this plea has considerable weight, considering the numerous variations in the nomenclature used to describe this component.

WADA's Explanatory Notes on the 2011 Prohibited List[6] state:

> *The stimulant 'methylhexaneamine' (which may be described, like many other substances, by other chemical names) is now included in the Prohibited List as a Specified Substance. This substance is now often marketed as a nutritional supplement and may frequently be referred to as 'geranium oil' or 'geranium root extract'.*

This statement comes from a general belief that geraniums contain methylhexaneamine based on what appears to be a single report in the literature.[7]

One of the attempts to try to conceal the presence of this compound is to refer to its presence under the name Geranamine and imply that it comes from geranium oil. The concern that geranium oil may contain methylhexaneamine has also been raised and so any product listing geranium oil could be used as a defence.

Geranium oils appear to be mainly obtained from *Pelargonium graveolens* which is a popular species of the geranium plant used in the production of geranium oil. Extraction of the oil can be performed by steam distillation or a cold-pressed process. A previous study of the chemical components in geranium oil by Ping *et al.* showed the presence of methylhexaneamine in geranium oil with a relative abundance of 0.66%.[7] This is the only such reference and is often quoted when referring to methylhexaneamine. It is apparent that few have read this paper critically!

A two-dimensional gas chromatography-mass spectrometry (GC-MS) study of *Pelargonium graveolens* essential oil by Shellie and Marriott identified 65 compounds.[8] Of the 65 compounds identified, none was found to correspond to methylhexaneamine. The authors of this study used a 30 m HP5 column for the first dimension and a temperature programme starting at 40°C. Under these conditions, the authors would have had no difficulty in observing methylhexaneamine, if it was present in the oil.

The aim of this short paper is to determine the presence/absence of methylhexaneamine in a selection of geranium oils by GC-MS compared to authentic standard. Geranium oils from companies which had been extracted from various species by cold-pressing or steam distillation procedures were purchased. It should be noted that methylhexaneamine is a volatile substance and is likely to have been lost through a steam distillation extraction, so cold-pressed material had the best chance of retaining this compound. The products used are shown in Table 1. A further product – Kaloba root

* *Correspondence to: Angelo Lisi, National Measurement Institute, 1 Suakin St., Pymble NSW 2073, Australia. E-mail: angelo.lisi@measurement.gov.au*

National Measurement Institute, Pymble, NSW, Australia

Table 1. Geranium products studied

Oils Analyzed	Manufacturer	Extraction process	Origin
Rose geranium oil	Sydney Essential Oil Co	Steam-distilled	France
Certified Organic Geranium Oil	Sydney Essential Oil Co	Steam-distilled	Egypt
Bourbon Geranium Oil	Sydney Essential Oil Co	Steam-distilled	Egypt
Geranium Oil	Sacred Essence	Cold-pressed	Egypt
Kaloba EPs 7630 Liquid	-	Ethanol extract	New Zealand

extract (from *Pelargonium sidoides*) obtained from New Zealand (via Drug Free Sport New Zealand) – was also studied, as it, too, was claimed to possibly contain methylhexaneamine.

Other supplements with stimulants as an ingredient were also studied in order to identify the main component. One of these supplements, Phenadrine, was used for an administration study as it contained both methylhexaneamine and phenpromethamine.

Experimental

Chemicals

Geranium oils were obtained from the manufacturers (Table 1) via Internet purchases. Methylhexaneamine (1,3-dimethylpentylamine), pentafluorbenzylchloride (PFBCL), and R(+)-α-methylbenzylamine were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). t-Butylmethyl ether (TBME) and hexane were purchased from Merck (Kilsyth, Victoria, Australia), and ethyl acetate from Mallinckrodt Chemicals (Mulgrave, Victoria, Australia). The supplements Adrenaline, Lipo 6 Black, Bang, and Phenadrine were obtained from Bodybuilding.com via Internet sales.

Analytical method

To the geranium oil or supplement (1 ml or 1 g) in a screw cap test tube was added 1 ml of 1 M hydrochloric acid, 100 μl of a solution of diphenylamine in methanol (100 μg/ml) and 5 ml of TBME. As a quality control, a further 1 ml aliquot of each oil was spiked with 200 μl of methylhexaneamine in methanol (100 μg/ml) and treated the same way. The mix was shaken on a rotary mixer for 30 min then centrifuged for 5 min. The TBME layer was discarded and the aqueous layer extracted twice more with TBME. To the resulting water layer was added an internal standard R(+)-α-methylbenzylamine (15 μg/ml), 300 μl of 6 M potassium hydroxide, 5 ml hexane and 2 μl of PFBCL. After shaking for 20 min and separation of the layers, the hexane layer was removed and evaporated to dryness. The residue was reconstituted with 200 μl of ethyl acetate and analysed by GC-MS in full-scan mode.

The analysis were conducted on a Agilent 6890 GC system coupled to 5973 MSD with an Agilent HP Ultra 2 (17 m x 0.2 cm x 0.25 μm) column with a 2 μl injection (split ratio 8:1). The injector temperature was set to at 260 °C with 15.0 psi column pressure and the temperature program from 87°C (1.5 min delay) to 160°C at 20°C/min then to 310°C at 35°C/min and held for 1.0 min. The mass spectrum was obtained in full scan mode from *m/z* 40 to 500 for confirmation with the use of *m/z* 238 for the semi-quantitative measurement of methylhexaneamine.

Administration study

The single oral administration of Phenadrine was carried out on a single volunteer with ethics approval from Southern Cross University. One capsule of the supplement was ingested and urine samples were collected between 0 and 29 h post-administration on an as-needed basis. The urine samples (5 ml) were made basic with 500 ul 6 M potassium hydroxide, internal standards 3-methyldiphenylamine and diphenylamine, 2.5 ml of t-butylmethyl ether and 2 g of anhydrous sodium sulfate were added and the mixture shaken for 20 min. After centrifugation, the upper layer was removed and analyzed by GC/NPD using an Agilent 689 GC with an Agilent HP Ultra 2 (17 m x 0.2 cm x 0.25 μm) column with a 2 μl injection (split ratio 5:1) and constant flow of 2.5 ml/min of helium, detector temperature 300°C and injector temperature 260°C and using a temperature programme: initial temperature 65°C then programme at 40°C /min to 310 then held at 310°C for 1.5 min. The samples were also subjected to the confirmation method similar to that described above and analyzed by GC-MS as pentafluorobenzyl derivatives. Semi-quantitative results were obtained by direct comparison to responses from calibration curves of standards using a routine GC/NPD procedure on urine extracts.

Results and discussion

Geranium oils

The analysis of the GC-MS and GC/NPD traces obtained from the geranium oils and the Kaloba root extract did not show the presence of any methylhexaneamine whereas the spiked traces showed good recovery of this substance. The trace (TIC) for the geranium oil extract spiked with methylhexaneamine is shown in Figure 1 and both enantiomers are clearly present. The spectrum for the derivatives of methylhexaneamine is shown in Figure 2; Figure 3 compares chromatograms from an oil sample spiked the methylhexaneamine to one of the geranium oils in this study.

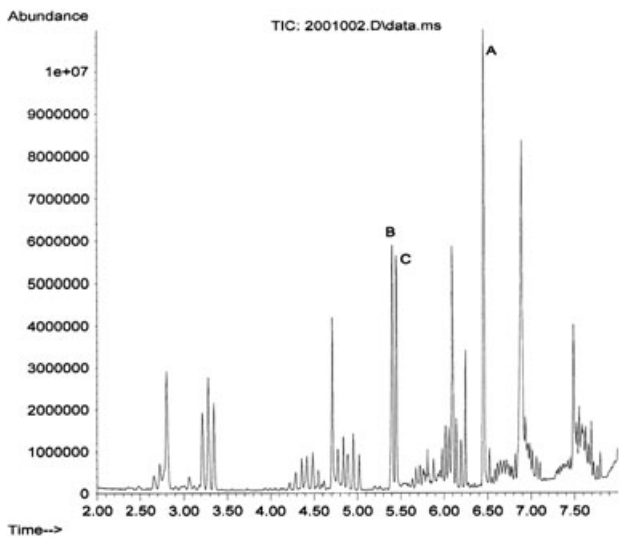

Figure 1. Full scan TIC of certified organic geranium oil spiked with methylhexaneamine. Peak A: Internal Standard, Peaks B: and C: the two isomers of methylhexaneamine.

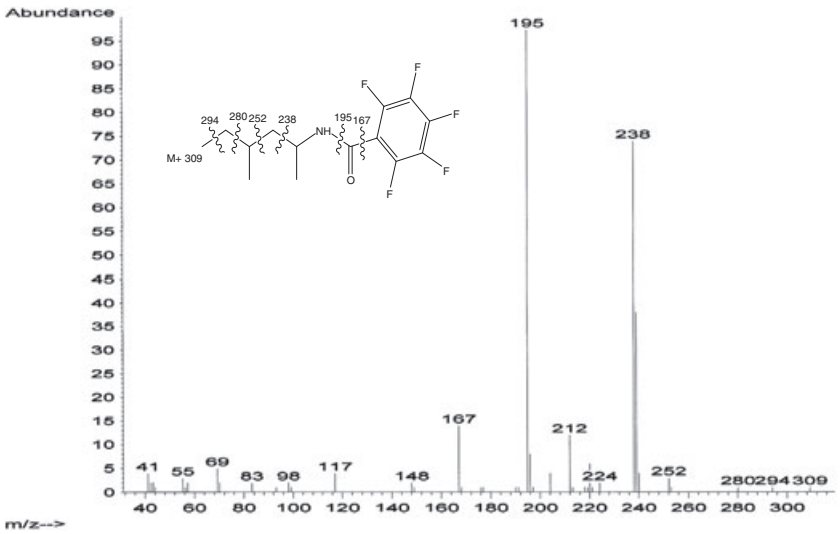

Figure 2. Mass spectrum of the pentafluorobenzoyl derivative of methylhexaneamine.

Methylhexaneamine was not found in any of the geranium oils described in Table 1. Inspection of the report by Ping *et al*. also clearly shows that their identification was incorrect or possibly incorrectly translated.[7] The table of components in Ping *et al*. shows compounds 30 and 31 (the latest eluting peaks from their chromatogram) as 2-hexanamide,4-methyl and 2-hexanamide, 5-methyl.[7] However, the Chinese translation has these as 4-methyl-2-hexanamine, and 5-methyl-2-hexanamine. This trace also has the compounds eluting late in the chromatogram whereas methylhexaneamine is very volatile and elutes very early requiring low GC starting temperatures which is inconsistent with this publication. Considering that the researchers simply ran the chromatogram then library searched each peak and presumably reported the best match without analysis of any standards, their assignments of structure are dubious. The spectrum of methylhexaneamine itself consists mainly of one ion *m/z* 44. Since very many substances possess a spectrum similar to this and this ion is of poor diagnostic value, it is reasonable to assert that the assignment was incorrect. The paper also performs quantification based on the TIC integration without calibration to any standards so their quantitative values will also have a very large uncertainty.

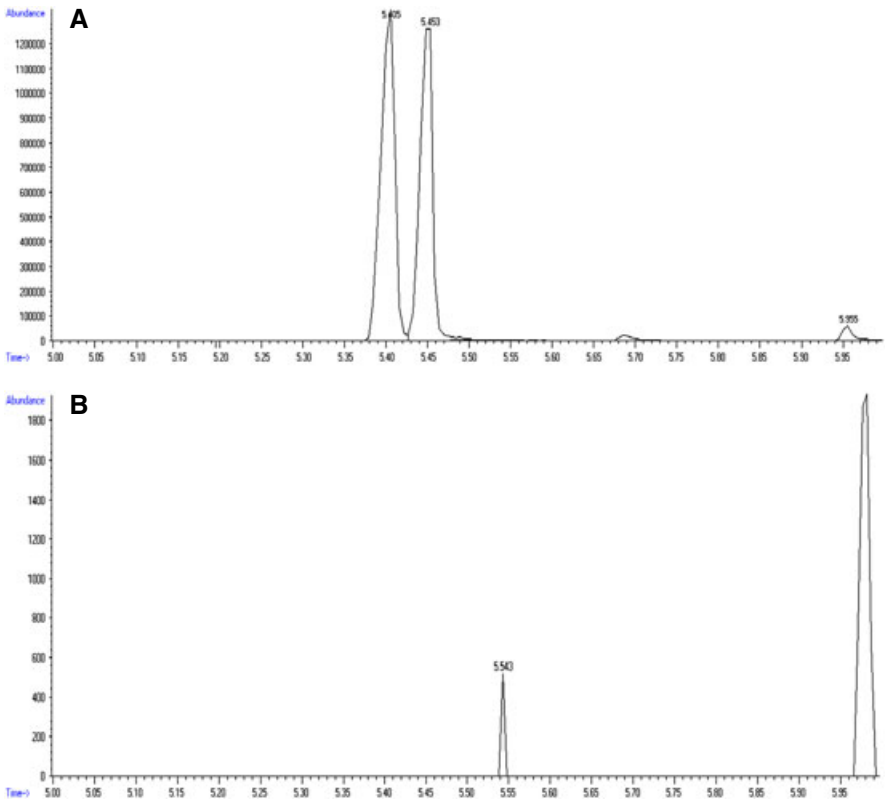

Figure 3. Extracted ion chromatograms for *m/z* 238 showing methylhexaneamine as the pentafluorobenzoyl derivative in a spike oil sample (A) compared to a certified organic geranium oil (B).

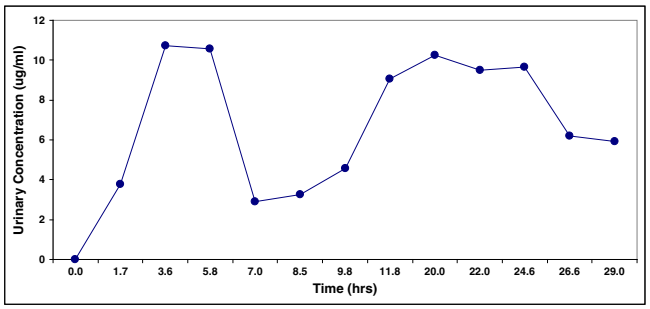

Figure 4. Methylhexaneamine urinary concentration profile over 29 hour excretion study, GC-NPD using a 4-point calibration at 2, 4, 8 and 12 ug/mL.

Supplements

Four supplements – Adrenaline, Bang, Lipo 6 Black, and Phenadrine – were analyzed and the stimulant ingredients were identified as methylhexaneamine (in Adrenaline), caffeine (in Bang), α and β-phenethylamine (in Lipo 6 Black), and methylhexaneamine and phenpromethamine (in Phenadrine). This is interesting, considering that the labels indicated they had mixtures of all these ingredients!

The excretion study for the supplement Phenadrine was undertaken to provide an excretion urine set for methylhexaneamine. Methylhexaneamine is easily detected as the parent compound in the urine and Figure 4 gives the time course for the excretion of a single subject over a 29-h period. The concentration of methylhexaneamine is not corrected for specific gravity but the data shows that it remains very high for more than 29 h (>3 ug/ml) which would allow it to be detected for several days if values above 50 ng/ml are reported.

Conclusion

These studies as well as closer scrutiny of the original publication by Ping *et al.*[7] show that geranium oils do not contain methylhexaneamine and the use of the name Geranamine for this compound appears to have been a marketing ploy which has resulted in a large number of athletes returning adverse findings. The excretion of methylhexaneamine occurs over an extended period with high levels still occurring after 29 h.

Other stimulants used in supplements can also cause adverse findings and the status of compounds such as α and β-phenethylamine as doping agents needs to be determined.

Acknowledgements

Investigations into metabolism and detection in urine of new designer steroids found in supplements are supported by the Australian Government through the Anti-Doping Research Program of the Department of Prime Minister and Cabinet. We thank Drug Free Sport New Zealand for assistance in obtaining Kaloba.

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