

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

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Methylhexaneamine (MHA) has been marketed in dietary supplements based on arguments that it is a constituent of geranium (*Pelargonium graveolens*) leaves, stems, roots or oil, and therefore qualifies as a dietary ingredient. The purpose of this study is to determine whether *P. graveolens* plant material (authenticated) or its oil contains detectable quantities of MHA. Two analytical methods were developed for the analysis of MHA in *P. graveolens* using gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. The results were further confirmed using liquid chromatography–high-resolution mass spectrometry. Twenty commercial volatile oils, three authenticated volatile oils and authenticated *P. graveolens* leaves and stems (young and mature, and fresh and dried) were analyzed for MHA content. In addition, three dietary supplements containing MHA that alleged *P. graveolens* as the source are analyzed for their MHA content. The data show that none of the authenticated *P. graveolens* essential oils or plant material, nor any commercial volatile oil of Pelargonium (geranium oil) contain MHA at detectable levels (limit of detection: 10 ppb). The dietary supplements that contained MHA as one of their ingredients (allegedly from geranium or geranium stems) contained large amounts of MHA. The amounts of MHA measured are incompatible with the use of reasonable amounts of *P. graveolens* extract or concentrate, suggesting that MHA was of synthetic origin.

Introduction

Pelargonium and Geranium are two distinct genera of the same family of plants. Oddly enough, pelargonium oil is commonly referred to in commerce as geranium oil. The oil from pelargonium is used extensively in aromatherapy, perfumery and cosmetics, while the oil from true geranium is used in herbal medicine (1).

A group of scientists from China reported a study on the chemical constituents of geranium oil (2) in which they isolated the leaves from *Pelargonium graveolens* by steam distillation (although there was no authentication of the plant material reported in the paper). They reported the major constituents of the oil as β -citronellol, geraniol, p-menthone, and linalool and their acetate and propionate esters, which is consistent with both the geranium and pelargonium genera (3, 4). They also reported traces of 4-methyl-2-hexaneamine (MHA; Figure 1A) at a level of approximately 0.6% by weight, along with other terpenes, sesquiterpenes and other aromatic and aliphatic compounds.

Chemically, MHA (CAS 105-41-9) is a simple aliphatic amine. MHA has many chemical names, including dimethylamylamine, 1,3-dimethylpentylamine, 2-amino-4-methylhexane and 4-methyl-2-hexylamine. Pharmacologically, it is classified as an α_1 -adrenergic agonist, and has been shown to be two hundred-fold less potent than *l*-epinephrine as a vasopressor in dogs, but with a much longer duration of action (5, 6) It was patented (7) and submitted to the Food and Drug Administration (FDA) for approval as a decongestant in the 1940s. The compound had an approved new drug application (NDA) and was sold as an over-the-counter drug until 1983. MHA has been reported as an active ingredient in party pills in New Zealand, where it has replaced 1-benzylpiperazine. One case report documents a cerebral hemorrhage in a 21-year-old after ingestion of MHA (8). Due to its purported stimulant effects and health risks, the Canadian Ministry of Health has clarified that MHA is a drug under their regulatory system (9). After four adverse events requiring emergency care, the New Zealand Health Ministry is also considering officially scheduling the drug (M. Heffernan, Health Ministry of New Zealand, personal communication; 10). In the United States and elsewhere, MHA is increasingly found in nutritional supplements such as weight loss and exercise stimulant supplements. In many cases, the product lists geranium oil or some part of the geranium plant on the content label, and in a few cases, the label lists the primary ingredient as MHA. Synthetic MHA can also be purchased in bulk from several chemical suppliers, and can be purchased in small quantities in its pure form over the internet.

The World Anti-Doping Agency has added MHA to the 2010 prohibited list (11). MHA has resulted in many reported doping cases (12–14) involving Indian, Nigerian and US athletes, presumably due to consumption of dietary supplements containing MHA.

Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), dietary supplements in the US that contain herbs and other botanicals and their constituents, extracts or concentrates may be legally sold as (or in) dietary supplements. Therefore, if MHA 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. However, 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 (15). The latter process requires the manufacturer to submit data to support the safety of the compound under the conditions of use. Because MHA is a substance prohibited in many sports, and because it is a stimulant that

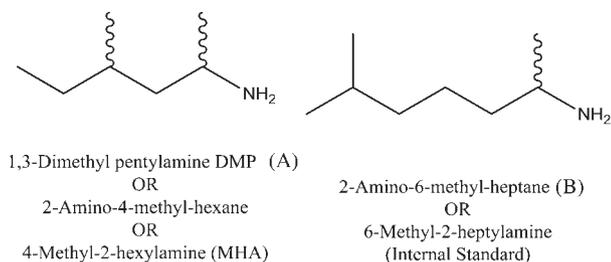


Figure 1. Structures of MHA and the 6-methyl-2-heptylamine IS.

reportedly carries significant health risks (8, 14), it was crucial to determine whether MHA could indeed be detected in *P. graveolens* plant material or essential oil.

Materials and Methods

Authenticated plant material

Authenticated *P. graveolens* leaves and authenticated *P. graveolens* oil (essential oil) were obtained from the Indian Institute of Integrative Medicine (IIIM). Authentication was provided by Dr. Y.S. Bedi, Head of the Plant Biotechnology Division, IIIM, Council of Scientific and Industrial Research (Jamun, India). In addition, *P. graveolens* leaves and stems were obtained from the medicinal plants garden of the National Center for Natural Products Research (NCNPR), School of Pharmacy, University of Mississippi. The plants were authenticated by Dr. Aroona Weerasooriya, Research Scientist (botanist) at NCNPR. Fresh leaves were used to prepare pelargonium volatile oil by steam distillation. The yield of the oil was 0.16% of the weight of the fresh leaves. All authenticated samples (plant material and volatile oil) were analyzed for MHA by gas chromatography–mass spectrometry (GC–MS), liquid chromatography–tandem mass spectrometry (LC–MS–MS) and liquid chromatography–high-resolution mass spectrometry (LC–QTOF–MS) methods.

MHA standard

MHA (Figure 1A) standard used in this study was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO), Catalog #S432989.

Internal standard

2-Amino-6-methylheptane (Figure 1B), (Sigma–Aldrich, Catalog #D16,129-2) was used as the internal standard (IS).

Chemicals and solvents

All chemicals and solvents used in the analysis were of ACS grade and were obtained from Fisher Scientific or VWR.

Commercial Pelargonium oils (geranium oils)

Samples were obtained from different sources over the period of January to May, 2011. These included different samples of *Pelargonium graveolens* (geranium) oils, one sample of

Pelargonium maculatum oil and one sample of *Pelargonium odorantissimum* oil.

Other commercial products

Three commercial dietary supplements were purchased from GNC.com on December 1, 2010, and analyzed for their content of MHA. Product A and Product B claimed to contain “dimethylamylamine (geranium [stems]),” both within a proprietary blend. Product C claimed the presence of MHA without reference to the source of the substance.

GC–MS analysis

Extraction and derivatization procedure for Pelargonium essential oil samples

A liquid–liquid extraction procedure was used for the extraction of MHA from the essential oil samples. IS (10 μ L of 10 μ g/mL) was added to an aliquot of the essential oil (100 μ L), and the mixture was dissolved in 2 mL of hexane with vortexing. The solution was then extracted with 2 mL of 1 N HCl. The hexane layer was removed and discarded and the acidic layer was extracted with an additional 1 mL of hexane, which was again separated and discarded. The aqueous acid layer was then adjusted to pH 9–10 with 10 N KOH and extracted with 3 mL of methylene chloride (DCM). The organic layer was transferred to a clean screw-capped conical tube and the solvent was evaporated to approximately 1 mL. To the DCM layer was added 100 μ L of heptafluorobutyric anhydride (HFBA), the tube was capped and then secured at room temperature for 1 h, after which 200 μ L of 2 N NaOH and 1 mL of 1.5 M sodium carbonate buffer were added while vortexing. After 5 min at room temperature, 200 μ L of chloroform was added and vortexed. The organic layer was then transferred to an autosampler vial for analysis on the GC–MS system.

Extraction of *P. graveolens* and related plant material

The plant material (1 g) was extracted with 5 mL of 0.1 N HCl–methanol. The solvent was evaporated and the residue was then partitioned between 1N HCl (1 mL) and DCM (2 mL). The acidic layer was separated and adjusted to pH 9–10 using 10 N KOH, extracted with DCM and the extract was treated in the same manner as described for the oil samples.

Samples were extracted without the addition of IS to ascertain whether MHA was present and to show that no interferences were present at the IS retention time. If a peak corresponding to MHA was found in the extract, the sample was to be re-analyzed with the IS for quantification. For samples that did not contain a peak corresponding to MHA, the oil or plant material was spiked with MHA at 1 ppm for the oils or 0.1 ppm for plant material and then extracted in the presence of IS to demonstrate MHA recovery by the extraction method.

The extraction efficiency was determined by comparing the area ratio of MHA to IS in samples extracted with IS added after extraction to the area ratio of MHA to IS in which both MHA and IS were added before extraction. This showed an extraction efficiency of approximately 35%.

Instrumentation

An Agilent 7890A GC interfaced to an Agilent 5975C MSD was used for the analysis. The analytical column was an Agilent J&W DB-5MS (25 m × 0.2 mm id; 0.33 μm df; PN: 128-5522), operated under programmed temperature conditions starting at 100°C (2 min) to 115°C at 2.5°C/min. (0.5 min hold at 115°C), and then programmed to 200°C at 70°C/min with 0.5 min hold at the final temperature. The mass spectrometer was operated in the selected ion monitoring (SIM) mode. The ions monitored for the heptafluorobutyrate (HFB) derivative of MHA were m/z 240, 296 and 282 (Figure 2), and those of the HFB derivative of the IS were at m/z 240, 282 and 310. The dwell time for each ion was 50 msec.

Validation of the procedure

A three-point curve (5, 10 and 25 ppm) was used as the calibration curve for routine analysis. Solutions were prepared at 0.1, 1, 2.5, 5, 10, 25, 50, 100 and 150 ppm to establish the characteristics of the assay. The limit of detection (LOD) for MHA was determined to be 0.1 ppm, the limit of quantitation (LOQ) was 2.5 ppm and the upper limit of linearity (ULOL) was 100 ppm. In addition, two controls were prepared from an essential oil previously shown to contain no MHA. The oil was spiked with MHA at approximately 2 and 8 ppm. The controls were analyzed in six replicates to determine the reproducibility of the assay. The intermediate precision of the method was 2.5% and 5% at 2 and 8 ppm, respectively. The accuracy of the method, based on analysis against the nominal concentration of the controls, was 75.5% and 85.7% at 2 and 8 ppm, respectively.

LC-MS-MS analysis

Extraction procedure

The same extraction procedure used for the preparation of plant material and oil samples for GC-MS analysis was used, except that the DCM layer was evaporated and 100 μL of methanol 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.

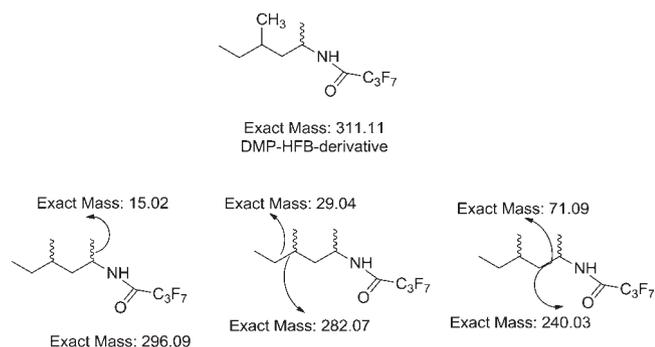


Figure 2. Proposed electron ionization fragmentation pattern of the HFB-derivative of MHA.

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 Qtrap 3200 with a turbo-ion ESI source operating in the positive ionization mode). Specific multiple reaction monitoring (MRM) transitions were monitored for each compound for maximum selectivity and sensitivity (Table I). Separation of MHA and its IS was achieved on a Synergi Hydro column (150 × 3.0 mm; 4 μm; 80 Å) from Phenomenex (Torrance, CA). A binary solvent gradient was used in which solvent A was composed of water with 0.1% formic acid and solvent B was acetonitrile with 0.1% formic acid. The solvent program consisted of a 2-min hold at 98/2 A/B, followed by a linear ramp to 15/85 A/B in 2 min. Solvent B was increased to 5/95 A/B over the next 0.5 min, and finally to 0/100 at 6 min. The column was washed with 100% B for 2 min and finally recycled to the initial conditions at 9 min. Data acquisition and processing was performed with the Analyst 1.5.1 software (Applied Biosystems, Foster City, CA).

Validation of the LC-MS-MS procedure

Multipoint calibration curves from 1–500 ng MHA/mL oil (1–500 ppb) were prepared for analysis. A three-point curve (10, 25 and 50 ppb) was used as the routine curve, with other points calculated based on this three-point curve to establish LOD, LOQ and ULOL. Solutions were prepared at 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 ppb to establish the characteristics of the assay. The LOD for MHA was determined to be 2.5 ppb, the LOQ was 2.5 ppb and the ULOL was 250 ppb. In addition, three controls were prepared from an essential oil previously shown to contain no MHA. The oil was spiked with MHA at 10, 25 and 50 ppb. The controls were analyzed and the within-batch and batch-to-batch coefficient of variation (CV) were calculated. The intra-assay CV was 15%, 8% and 4% at 10, 25 and 50 ppb, respectively. The intermediate assay CV was 5%, 6% and 6.5%, respectively.

High resolution LC-QTOF-MS method

Samples were prepared as described previously for the LC-MS-MS method. Chromatography was performed on an ACQUITY UPLC system (Waters Corp., Milford, MA) 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). The column temperature was maintained at 40°C. The analysis was achieved with gradient elution using acetonitrile (A) and water (B) (containing 0.05% formic acid) as the mobile

Table I

Compound-Dependent MS-MS Parameters Optimized for MRM Acquisition Mode*

Compound name	R _t (min)	M (amu)	Precursor ion (m/z)	Product ions MRM 1/2	DP (V)	CE (V)
MHA	4.19	115	116	T1: 116.1/56.9 T2: 116.1/41.0	36	15
6-Methyl-2-heptylamine (IS)	4.32	129	130	T1: 130.0/57.0	51	21

*Note: MRM 1/2 signifies the first (T1) and second (T2) transition; T1, quantifier; T2, qualifier; DP, declustering potential; CE, collision energy.

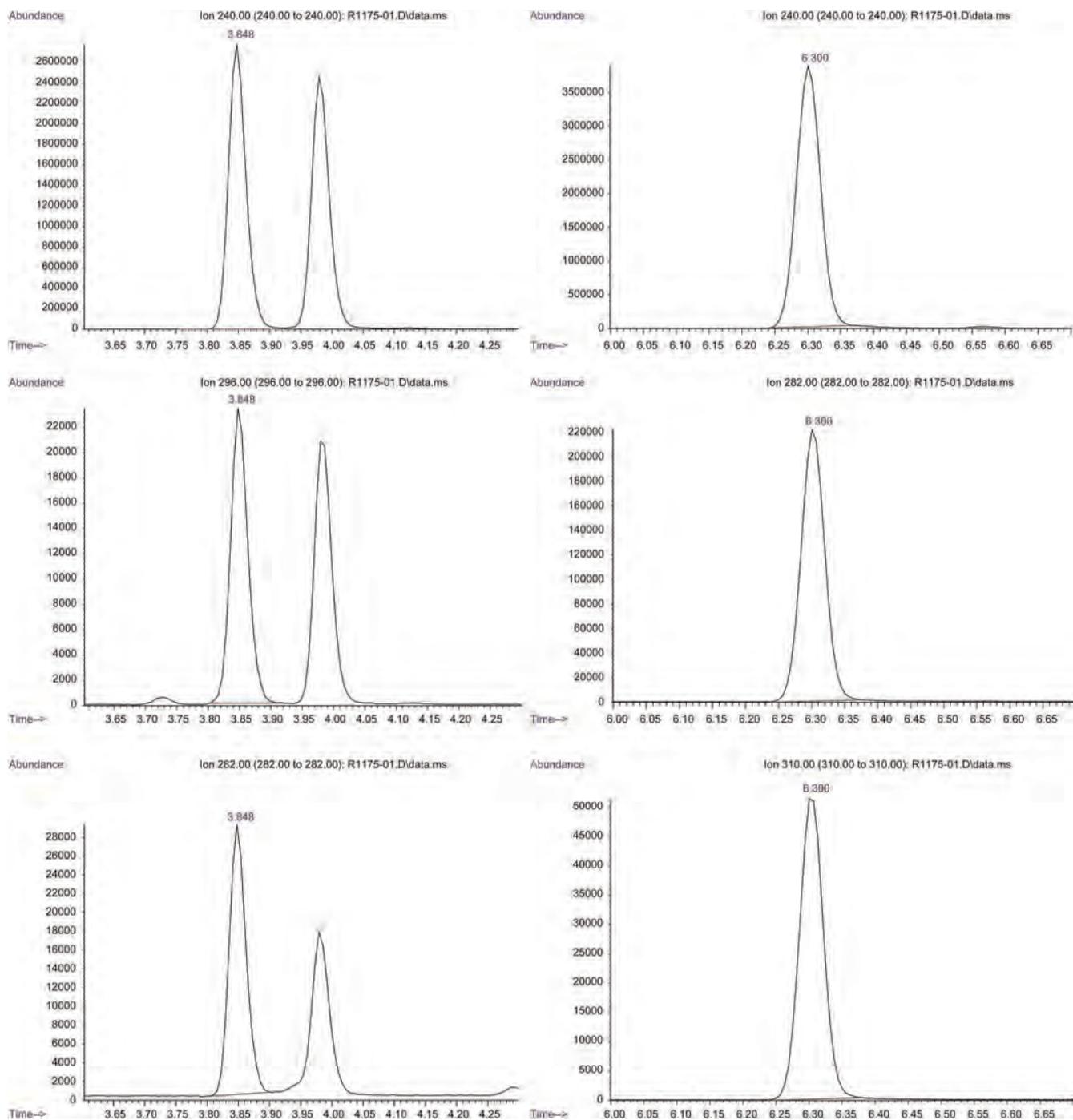


Figure 3. GC-MS selected ion chromatograms for MHA as the HFB derivative (1 μ g unextracted standard): IS MHA ions at m/z 240, 296 and 282 (top to bottom) are shown on the left, while those of IS-HFB (m/z 240, 282 and 310) are shown on the right.

phase at a flow rate of 0.25 mL/min. The gradient conditions were: 0–4 min linear from 5 to 70% A. The Waters ACQUITY Xevo G2 Qtof Mass Spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system via an electrospray ionization (ESI) interface. The ESI source was operated in 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). Accurate mass calibration for positive ESI 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 425.1825, 397.1876, 278.1141) at a concentration of 2 ng/ μ L and flow rate of 5 μ L/min to ensure accuracy during the MS analysis.

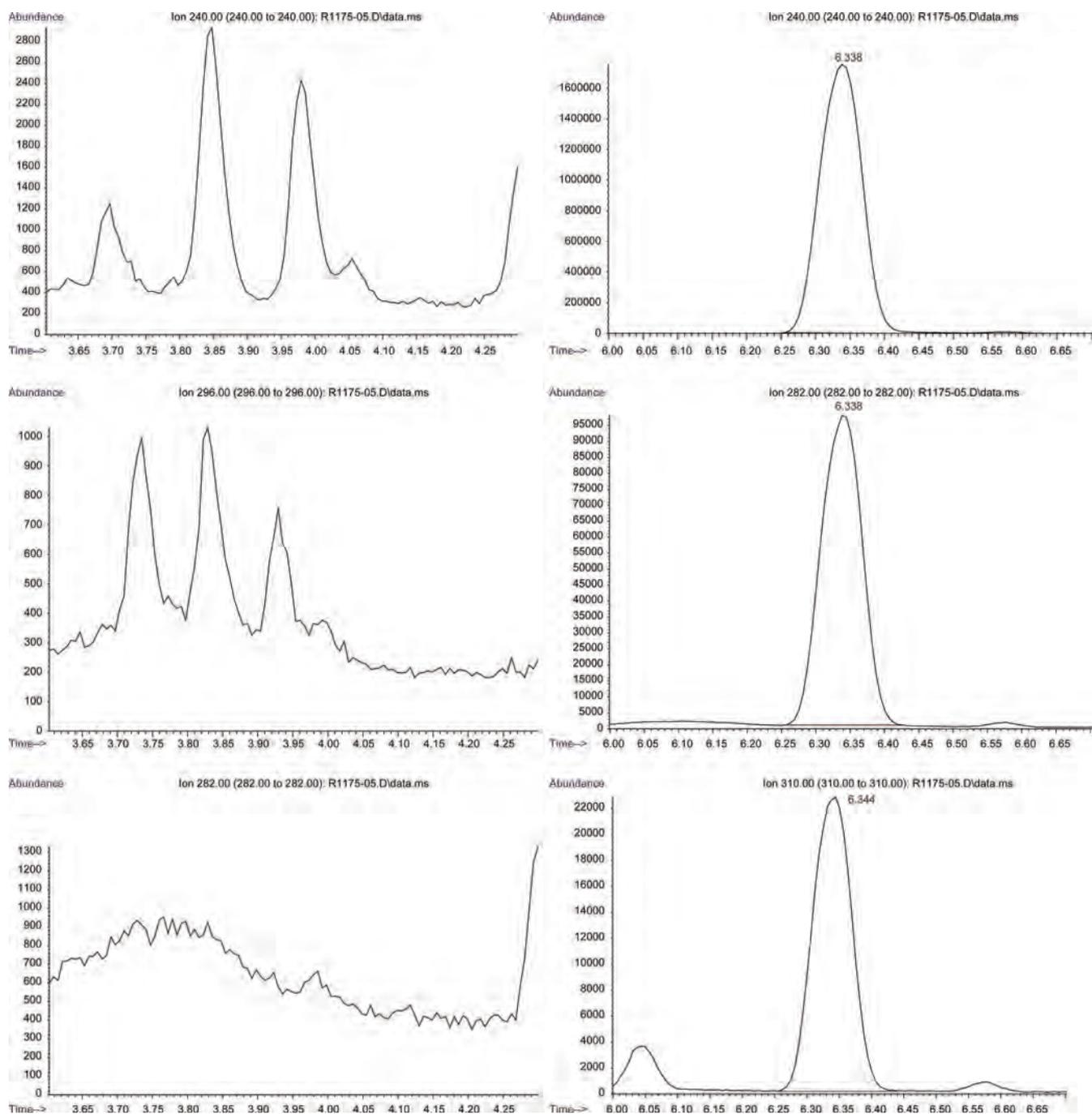


Figure 4. GC-MS selected ion chromatograms of an MHA-negative geranium oil control with IS ions for MHA-HFB (m/z 240, 296 and 282) are shown on the left, while those of IS-HFB (m/z 240, 282 and 310) are shown on the right.

The lockspray interval was set at 10 s and the data were averaged over 5 scans. The mass spectrometer was programmed to step between low energy (3 eV) and elevated energy (10–20 eV) collision energy on the gas cell, using a scan time of 1 s per function over 20–500 m/z . When data were acquired with MSE, 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. MSE data independent analysis provides accurate mass measurements of all detectable precursor and product ions, which is 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

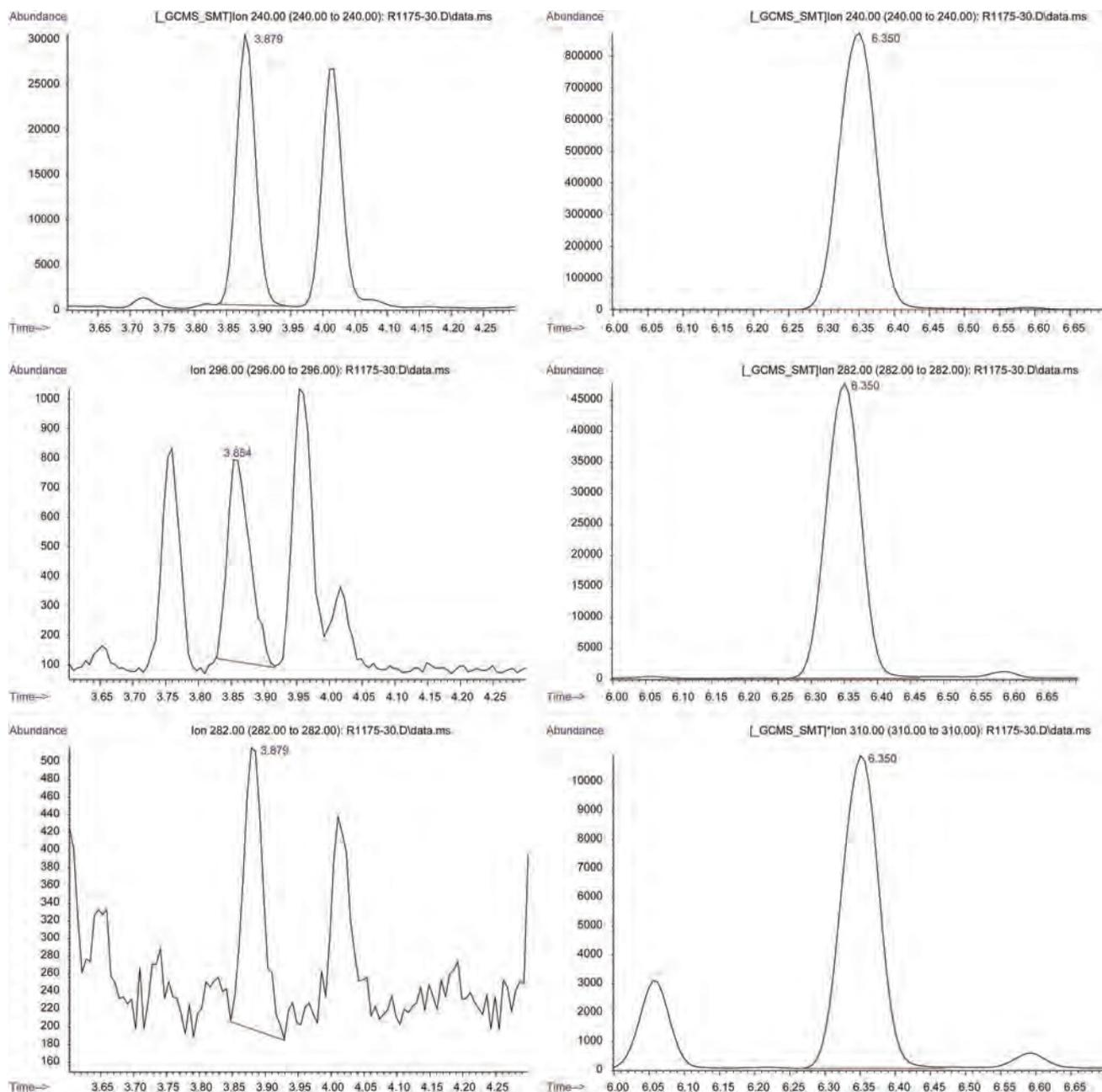


Figure 5. GC-MS selected ion chromatograms for a MHA negative geranium oil spiked at 0.1 ppm (100 ng/mL) MHA and IS ($1 \mu\text{g/mL}$): MHA-HFB ions at m/z 240, 296 and 282 are shown on the left, and those of IS-HFB (m/z 240, 282 and 310) are shown on the right.

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 LOD for this method was estimated at 10 ppb.

Results and Discussion

A procedure for the analysis of MHA in *P. graveolens* essential oil (geranium oil) and essential oils from related species was developed using GC-MS in the SIM mode. The method was

extended to the analysis of the plant's leaves by first extracting the leaves with 0.1 N HCl in methanol followed by processing the extract (after evaporation of the solvent) in the same manner as the oil. The method was found to have good sensitivity and reproducibility. The chromatographic peak height for the ion arising from the base peak in the mass spectrum (m/z 240) of the HFB-derivative of MHA for the 0.1 ppm concentration was readily apparent. One can easily determine the absence of MHA in a sample at a much lower level than 0.1 ppm. However, positive identification below 0.1 ppm was not possible because of the low intensity of the other qualifier ions. As a result, the LOD was reported at 0.1 ppm.

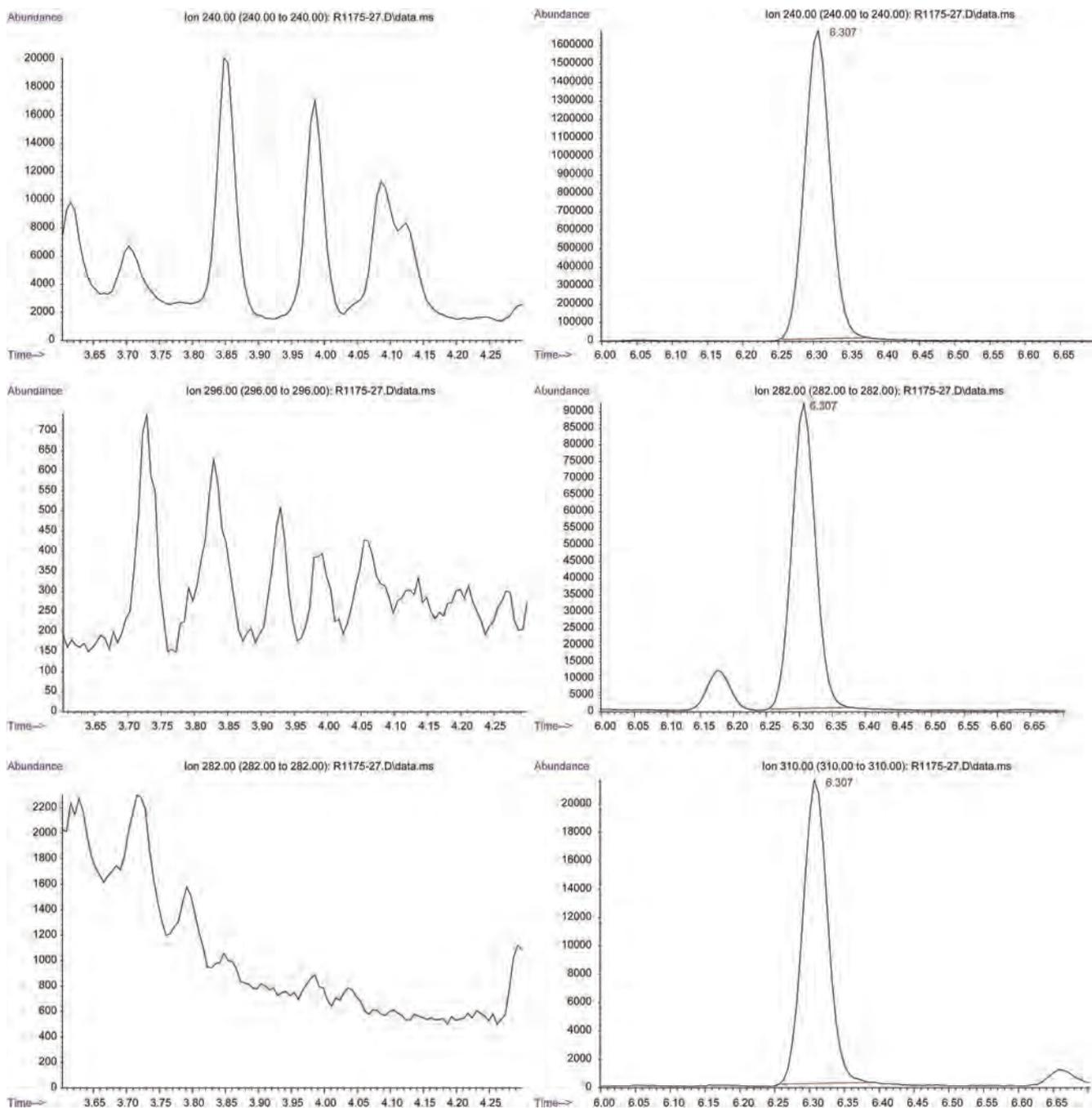


Figure 6. GC-MS selected ion chromatograms of MHA-HFB from the extract of authenticated *P. graveolens* plant material with IS. The ions for MHA-HFB at m/z 240, 296 and 282 are shown on the left, and those of IS-HFB (m/z 240, 282 and 310) are shown on the right. Note the absence of MHA.

Twenty commercial volatile oils, three authenticated volatile oils and as authenticated *P. graveolens* leaves and stems (young and mature, and fresh and dried) were analyzed following the procedure described previously without IS to make sure there were no interferences at the IS retention time. None of those oils or the plant stems or leaves contained detectable amounts of MHA (much less than the LOD). To prove that the absence of MHA in the oils and the plant material is not because of lack of extraction recovery, aliquots of samples of the oils and the plant material were spiked with 0.1 μg of MHA

that would represent 1 ppm for the oils (0.1 mL used for analysis) and 0.1 ppm for the plant material (1 g was used for analysis), and the spiked samples were analyzed in the presence of the IS. The results showed recovery of the spiked MHA and the IS, indicating that the absence of MHA in the samples is not because of lack of recovery in the extraction process. The GC-MS selected ion chromatograms (Figures 3 to 9) show representative chromatograms for an unextracted standard; a negative sample with the IS; an oil sample spiked at 0.1 ppm; extract of authenticated *P. graveolens* plant material with

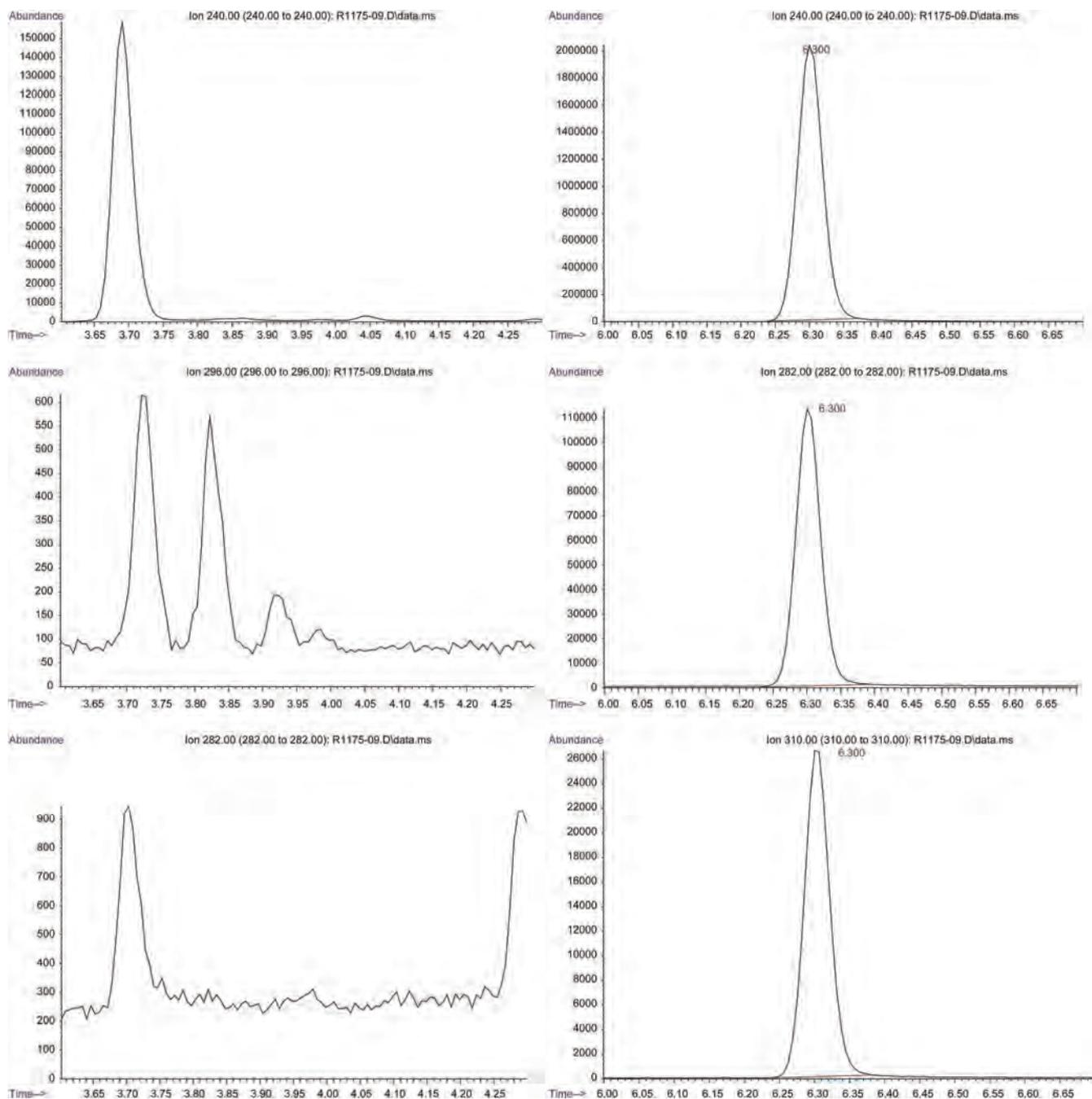


Figure 7. GC-MS selected ion chromatograms of MHA-HFB from an extract of authenticated *P. graveolens* oil with IS. The ions for MHA-HFB at m/z 240, 296 and 282 are shown on the left, while those of IS-HFB (m/z 240, 282 and 310) are shown on the right. Note the absence of MHA.

the IS, showing the absence of MHA; extract of authenticated *P. graveolens* oil with the IS, showing the absence of MHA; extract of 0.1 mg of a powdered commercial product alleging *P. graveolens* as the source of MHA; and the HFB derivative of the IS showing a small amount of MHA (<1%) at the retention time (R_t) of the high intensity ion at m/z 240.

Because of the relatively high LOD (0.1 ppm) of the GC-MS method, an LC-MS-MS method was developed for the analysis of MHA in *P. graveolens* oil and plant material. The method was highly sensitive (LOD and LOQ of 2.5 ppb), and therefore

offered confirmation that the samples shown by the GC-MS to be negative at the 0.1 ppm level were also negative at a forty-fold lower detection level. All samples were reanalyzed by this LC-MS-MS method. None of the oils were found to contain MHA above the LOD of the assay. Representative chromatograms from the LC-MS-MS analysis of calibrators, oil samples, plant material samples and three different commercial products containing MHA are shown in Figures 10 to 14.

The results of the GC-MS and LC-MS-MS analyses were further confirmed by subjecting the extracts of the oils and

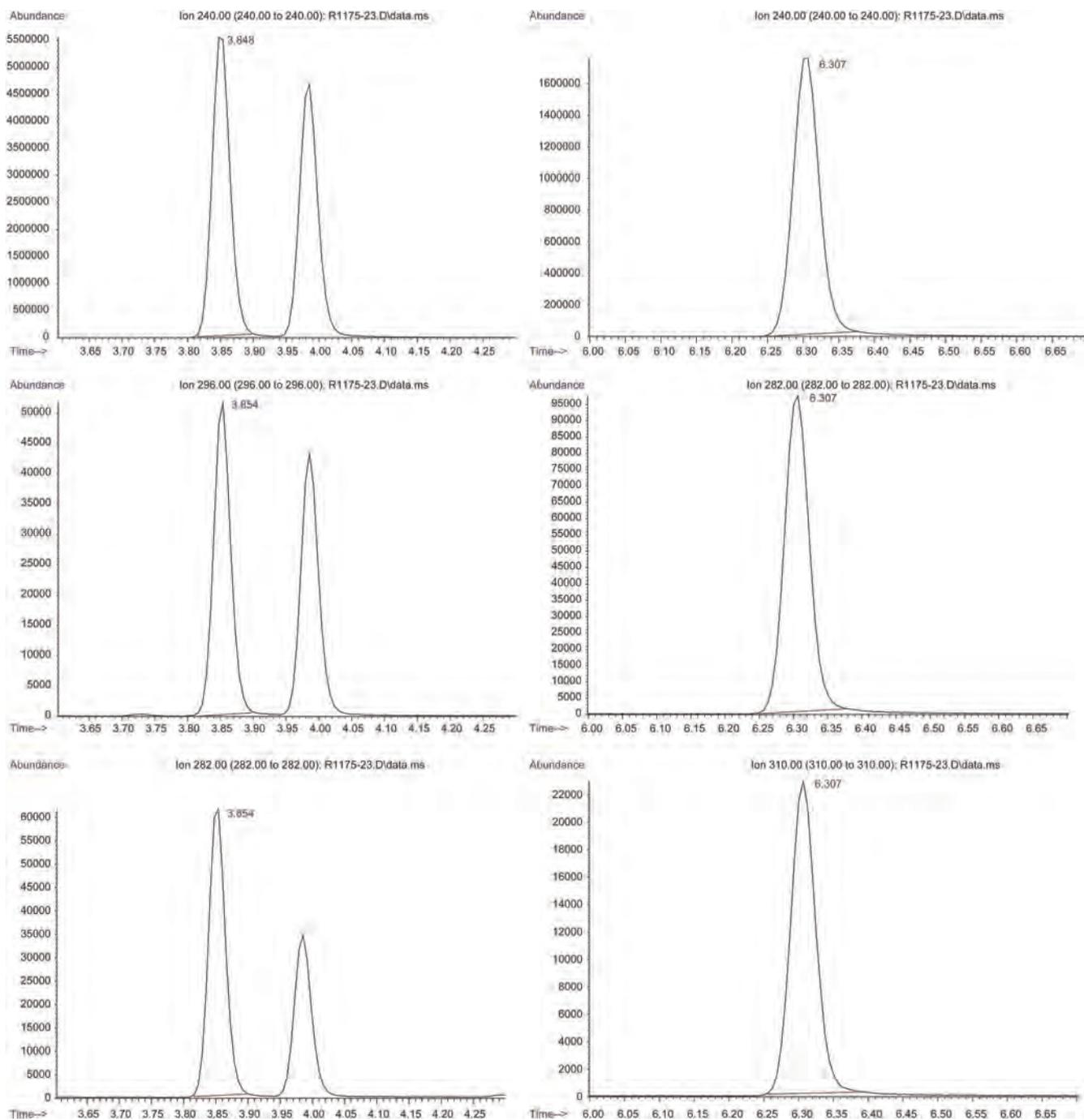


Figure 8. GC–MS selected ion chromatograms of the HFB derivative of the extract of 0.1 mg of a powdered commercial product alleging *P. graveolens* as the source of MHA. The MHA–HFB ions (m/z 240, 296 and 282) are shown on the left, while those of IS–HFB (m/z 240, 282 and 310) are shown on the right. Note the high level of MHA (calculated value of >10 mg MHA/g) powdered product.

plant material to high resolution LC–QTOF-MS analysis. Because this method had an LOD of 10 ppb, confirmation of the presence of MHA required concentration of ≥ 10 ppb. The analysis showed that all authenticated *P. graveolens* plant material, authenticated *P. graveolens* volatile oils and commercial geranium oil purchased on the open market were negative (less than 10 ppb) for MHA.

Table II shows a list of the different types of *P. graveolens* plant tissues and a variety of authenticated and commercial Pelargonium essential oils (geranium oils) analyzed for this study. The analysis of both young and mature leaves shows the absence of MHA at different stages of growth of the plant, while the analysis of fresh and dried tissues shows that no MHA is formed as a result of drying of the plant material. All of

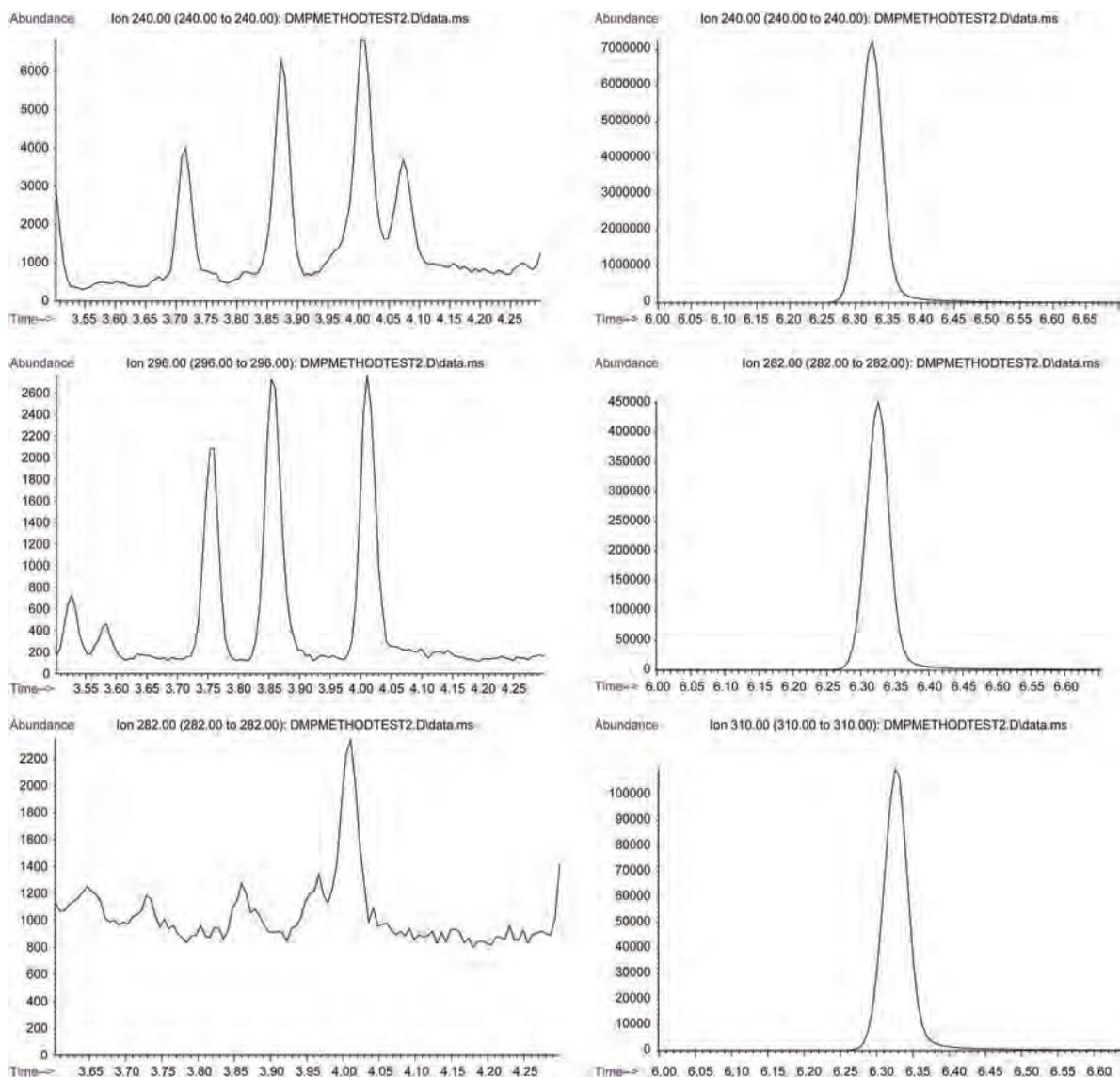


Figure 9. GC–MS selected chromatograms for the HFB derivative of the IS showing a small amount of impurity of MHA (<1%). Ions for MHA at m/z 240, 296 and 282 are shown on the left, while those for the IS (m/z 240, 282 and 310) are shown on the right.

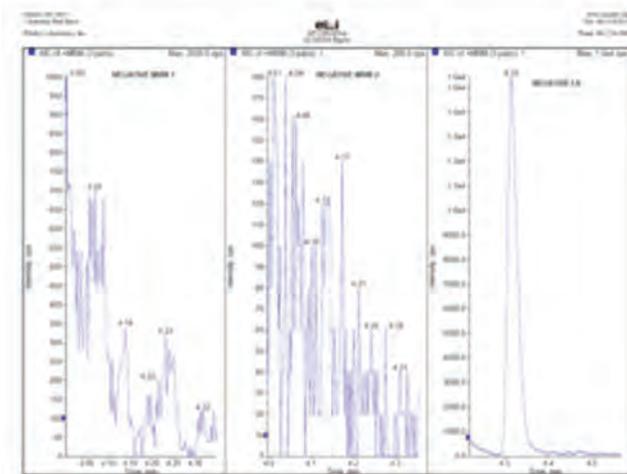
the commercially relevant geranium oils, including that extracted from *P. graveolens*, have been extremely well-characterized and we could find no peer-reviewed or commercial document establishing MHA as a constituent of any *Pelargonium* or geranium species (1, 3, 4). In fact, the only literature report showing *P. graveolens* volatile oil to contain MHA is that of Ping *et al.* (2). In their report, the authors indicated that GC–MS analysis in the full scan mode showed the presence of MHA at a level exceeding 5 mg/mL (i.e., ~0.6% of the oil). Viewing the GC–MS chromatogram in the paper, the peak for MHA is too small to see, and therefore it is unclear whether the spectrum represents a single compound. The authors indicated that the identification was based on library search without any indication about the quality of the match, MS spectrum comparison, or probability fit. Therefore, it is not possible to determine from the presented data whether the compound labeled as MHA is actually MHA. Because this is the

only publication suggesting that MHA is contained in *P. graveolens*, careful review indicates that the data do not support the conclusion that MHA is a minor constituent of geranium oil.

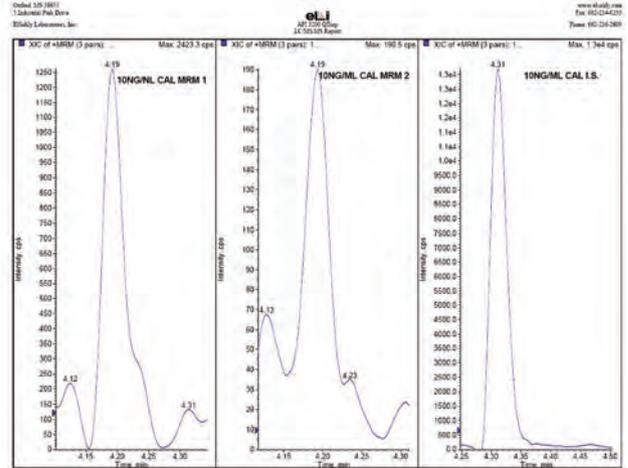
In contrast, the current study was specifically designed to detect MHA in oil or plant material, as opposed to generally characterizing the major constituents. We were not able to detect MHA in geranium oil at an amount of 10 ppb (the LOD for the LC–QTOF high resolution method) which is 0.5 million orders of magnitude below that previously reported (2).

Three dietary supplements were also analyzed by the procedures described previously. Product A claims on its label to contain “dimethylamylamine (geranium [stem])” as part of a proprietary blend. Product B claims that each capsule contains “dimethylamylamine HCl (geranium stem)” within a proprietary blend, in addition to 100 mg of caffeine. Product C claims on its label to contain “1,3-dimethylamylamine” as part of a proprietary blend. The amount of MHA measured in the products

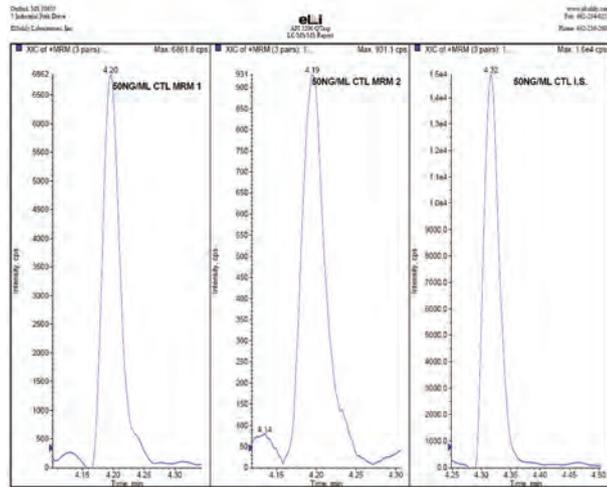
A



B



C



D

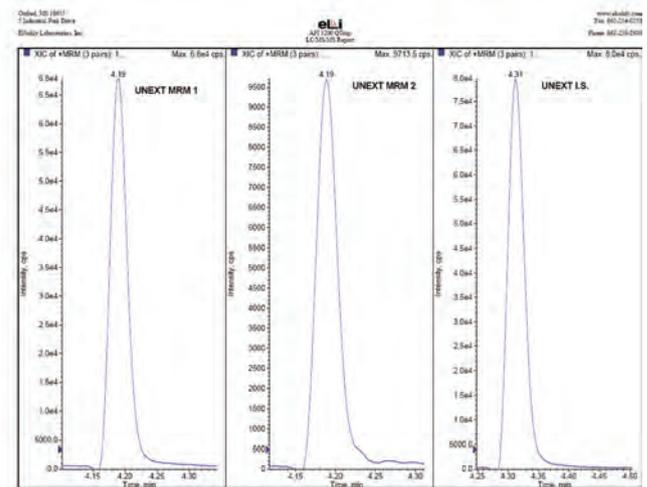
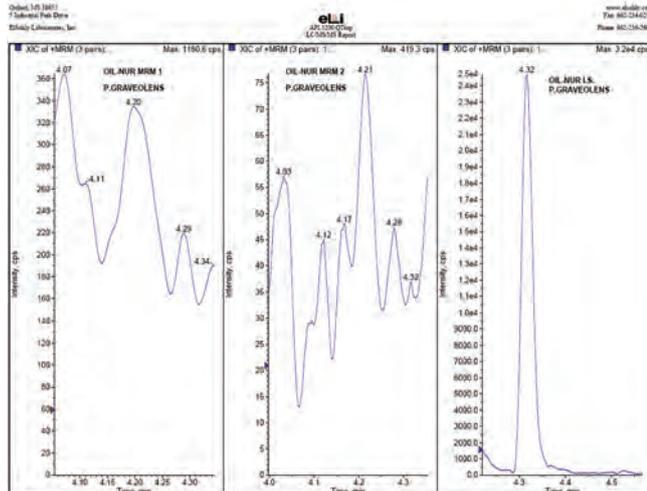


Figure 10. Chromatograms for MRM 1, MRM 2 and IS of: negative (A); 10 ng/mL calibrator (B); 50 ng/mL control (C); un-extracted MHA and IS at 50 ng/mL (D).

A



B

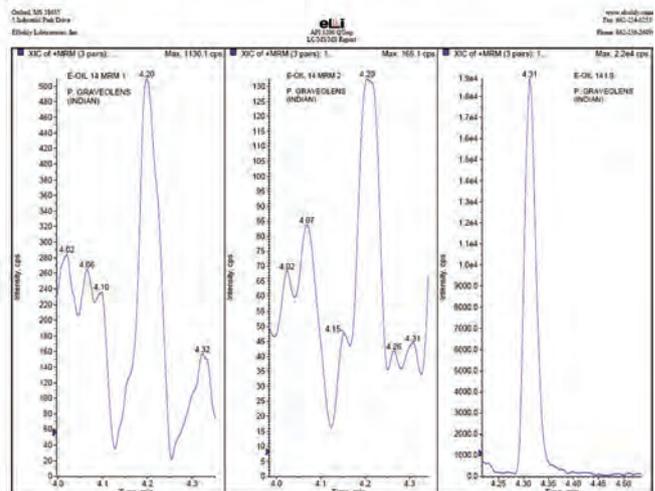


Figure 11. Chromatograms for: MRM 1, MRM 2 and IS of: oil extracted from *P. graveolens* (A); authenticated oil of *P. graveolens* from Srinagar (India) (B).

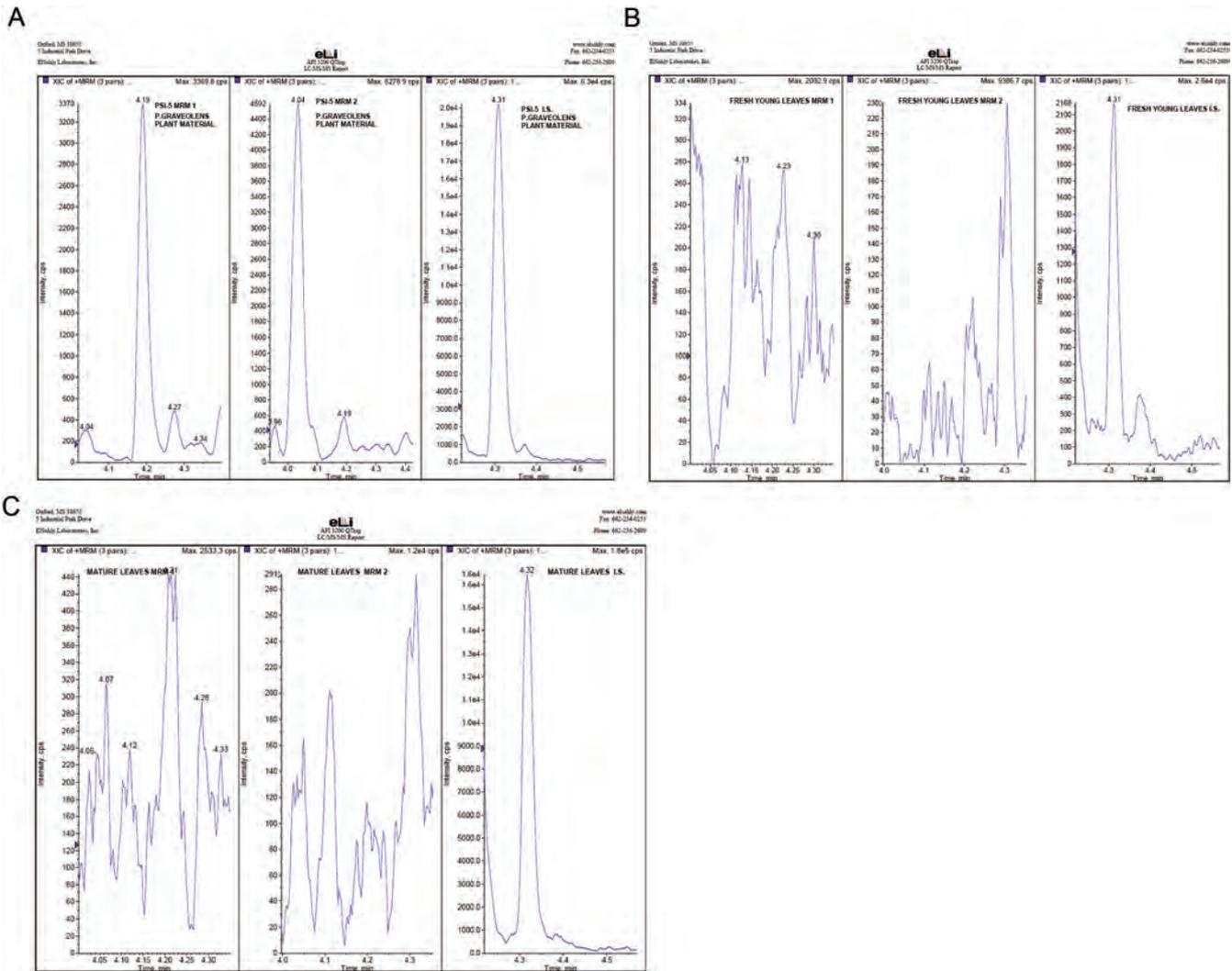


Figure 12. Chromatograms for MRM 1, MRM 2 and IS of: authenticated *P. graveolens* plant material from India (A); authenticated young leaves extract of *P. graveolens* from the Medicinal Plant Garden, NCNPR, University of Mississippi (B); authenticated mature leaves extract of *P. graveolens* from the Medicinal Plant Garden, NCNPR, University of Mississippi (C).

is shown in Table II. All of these products contained more than 1 mg MHA per g of dietary supplement. Each of the supplements contained more than 250 g of supplement material per bottle, which would require at least 250 mg of MHA per bottle of supplement.

The yield of geranium oil from the leaves of *P. graveolens* is 0.1–0.3%. If MHA were to be present in the oil at 0.6% (i.e., 6 mg MHA/mL oil), as reported (2), and assuming the leaves yield 0.1% oil, it would require approximately a kilogram of leaves to be harvested to prepare 6 mg of MHA. If we assume that a dietary supplement contains 10 mg MHA per capsule, then one bottle of 100 capsules would require the processing of 167 kg of *P. graveolens* leaves for 167 mL of geranium oil! The current study shows that MHA, if present at all, would be present at an amount less than 0.000001% in geranium oil, so the suggestion that MHA is part of a concentrate or extract from *P. graveolens* becomes even more difficult to believe.

A dietary supplement, according to DSHEA, is a product that is labeled as a dietary supplement and is not represented for use as a food or as a cure for any disease. DSHEA specifically granted manufacturers the ability to include in their dietary supplements “a concentrate, metabolite, constituent, extract, or combination of any ingredient” of a vitamin, a mineral, an herb or other botanical, an amino acid, or a “dietary supplement used by man to supplement the diet by increasing the total dietary intake.” DSHEA also establishes separate standards for the safety of dietary supplements. For NDI, defined as those marketed after October 15, 1994, or unless an ingredient has been “present in the food supply as an article used for food in a form in which the food has not been chemically altered,” the manufacturer must provide FDA with information, based on a history of use or other evidence of safety, supporting the conclusion that the product “will reasonably be expected to be safe.” In the case of MHA, the claim for MHA as a dietary ingredient is based squarely on the scientific validity of the report of

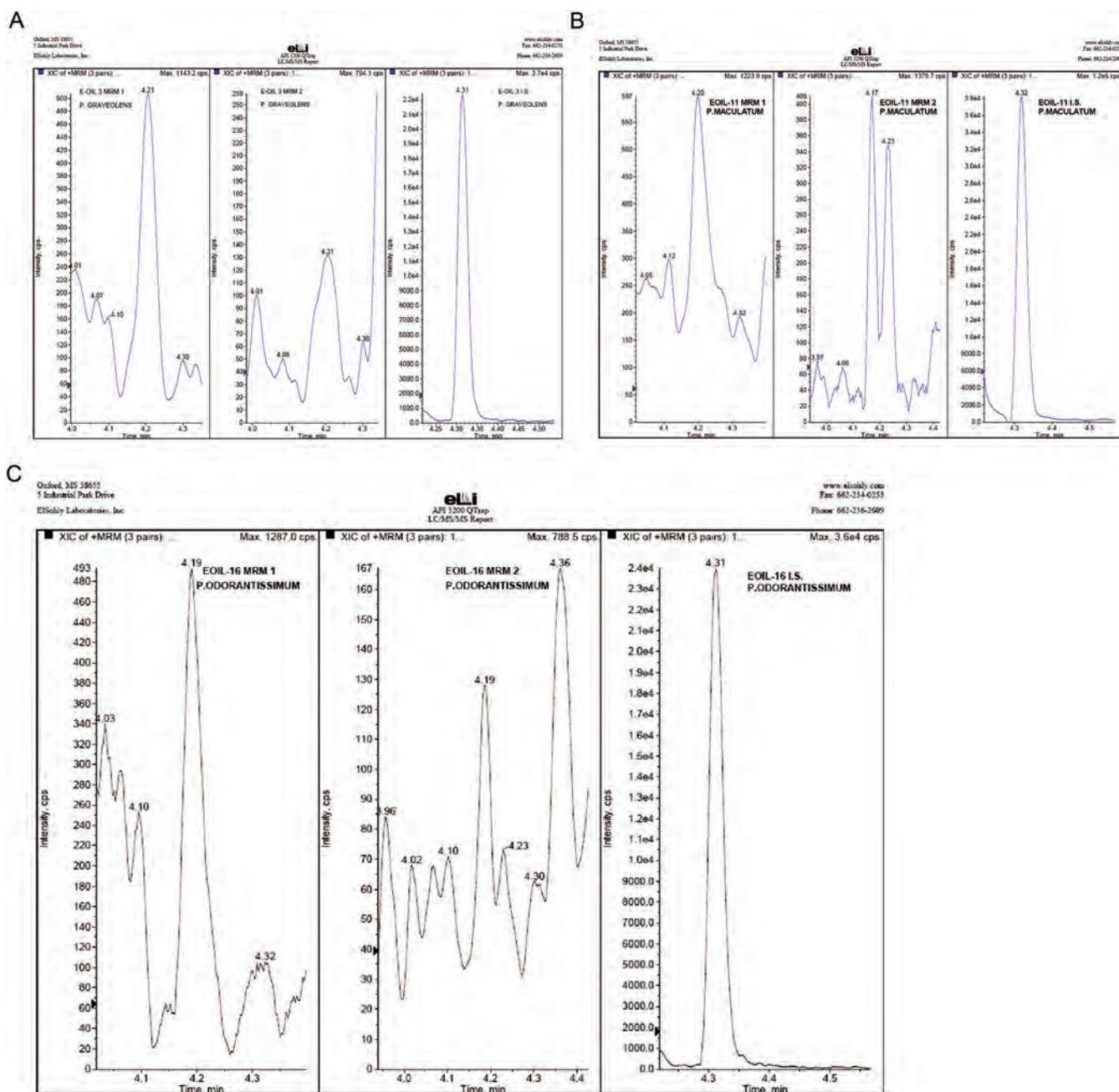


Figure 13. Chromatograms for MRM 1, MRM 2 and IS of: commercial oil of *P. graveolens* (A); commercial oil of *P. maculatum* (B); commercial oil of *P. odorantissimum* (C).

Ping *et al.* (2). It is also clear from the literature and the results presented here that it is not possible for geranium plant material, a dietary ingredient prior to 1994, to be the source of MHA in dietary supplements. Furthermore, had we detected MHA in geranium oil, or even if the presence of MHA is below our level of detection, such low levels could not possibly be sufficient to account for that MHA found in the dietary supplements tested in this study, or many other MHA-containing products on the market. Elementary mathematics establishes that the MHA in dietary supplements must be synthetic. Also, MHA was patented by Lilly in 1944, received FDA approval as a

decongestant, and was sold under the name Forthane (16). The FDA approval was withdrawn by request from Lilly in 1983 (17), and currently the name Forthane is used as a proprietary name for isoflurane.

There is reasonable cause for concern regarding the safety of MHA, given two published case reports in which ingestion of MHA resulted in severe adverse events, including cerebral hemorrhage (8, 18). A recent publication (19) reports that acute ingestion of MHA alone and in combination with caffeine results in an increase in systolic blood pressure and diastolic blood pressure without an increase in heart rate. We are aware

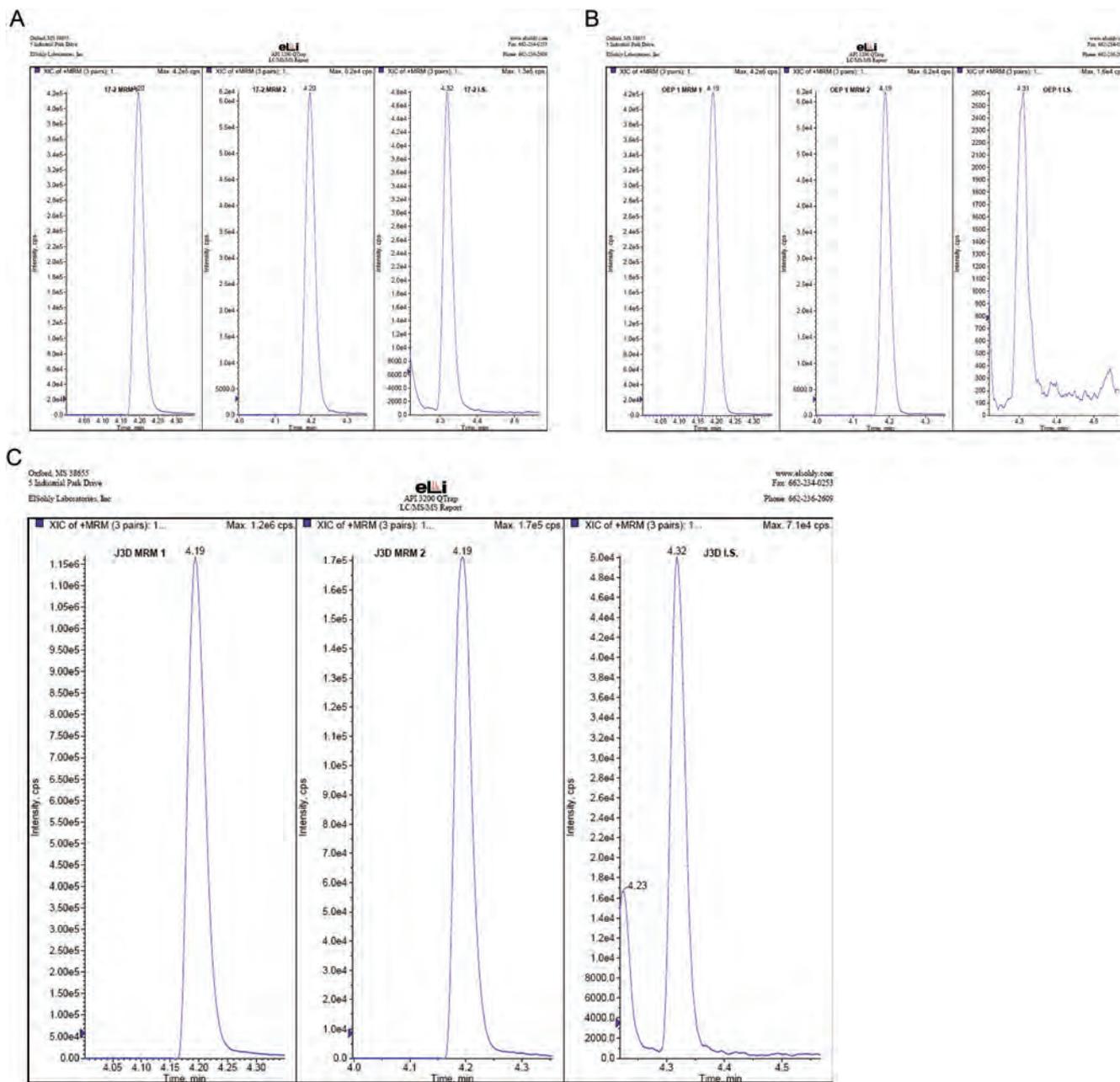


Figure 14. Chromatograms for MRM 1, MRM 2 and IS of three commercial powder samples containing MHA.

of several additional cases of toxicity in which the use of MHA was reported or MHA was found in the blood of the subject, the most severe of which reportedly resulted in coma and death (20).

Conclusion

Two sensitive and reliable procedures were developed for GC-MS and LC-MS-MS analysis of MHA in *P. graveolens* plant materials and volatile oils. None of the analyzed oils or the

plant material (young and mature, fresh and dried leaves and stems) showed any detectable level of MHA (< 0.1 ppm by GC-MS, < 2.5 ppb by LC-MS-MS and 10 ppb by the high resolution LC-QTOF). The absence of MHA was further confirmed by LC-QTOF-MS analysis. The procedures were used to analyze three commercial supplements containing MHA, two of which have listed *P. graveolens* as the source of MHA. All three supplements showed levels of MHA greater than 1 mg/g, suggesting that synthetic MHA was actually added to the products, rather than the result of the product's content of *P. graveolens*.

Table II

Concentration of MHA in Samples of the Authenticated Volatile Oil of *P. graveolens*, Authenticated Plant Material (Leaves and Stems), Several Commercial Oils and Three Products Alleged to Contain MHA as a Component of *P. graveolens* Oil

Authentic plant material		Concentration of MHA*	
Sample	Sample name	GC-MS	LC-MS-MS
PSI-5	<i>Pelargonium graveolens</i> (geranium), dried plant	ND	< 10 ng/mL
PSI-54-3A	Dried stem A	NA**	< 10 ng/mL
PSI-54-3B	Dried stem B	NA	< 10 ng/mL
PSI-54-3C	Fresh stem A	NA	< 10 ng/mL
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	NA	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 <i>P. graveolens</i>			
EOIL-14	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-15	<i>Pelargonium graveolens</i> (Geranium) UM Oil	ND	< 10 ng/mL
Commercial volatile oils			
EOIL-1	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-2	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-3	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-4	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-5	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-6	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-7	<i>Pelargonium graveolens</i> (Geranium Bourbon)	ND	ND
EOIL-8	<i>Pelargonium graveolens</i> (Geranium Egyptian)	ND	ND
EOIL-9	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-10	<i>Pelargonium graveolens</i> (Geranium)	ND	ND
EOIL-11	Wild Geranium; Herb Pharma	ND	< 10 ng/mL
EOIL-16	<i>Pelargonium odorantissimum</i>	ND	< 10 ng/mL
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	<i>Pelargonium odorantissimum</i> (Geranium oil)	ND	ND
PSI-20-4	Geranium oil	ND	ND
PSI-20-5	Geranium oil	ND	< 10 ng/mL
PSI-22	100% pure geranium	ND	< 10 ng/mL
PSI-25	100% geranium	ND	< 10 ng/mL
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: not detected (below LOD of 10 ng/mL or 10 ng/g); NA: not analyzed.

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References

- Lis-Balchin, M., ed. (2002) Geranium and Pelargonium. Medicinal and aromatic plants—Industrial profiles. Taylor and Francis, London.
- Ping, Z., Jun, Q., Qing, L. (1996) A study on the chemical constituents of geranium oil. *Journal of Guizhou Institute of Technology*, **25**, 82–85.

- Rao, B.R. (2002) Biomass yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium* species) as influenced by row spacing and intercropping with cornmint (*Metha arvensis* L.f. *piperascens* Malinv.ex Holmes). *Industrial Crops and Products*, **16**, 133–144.
- Rao, B.R., Kaul, P.N., Syamasundar, K.V., Ramesh, S. (2002) Water soluble fractions of rose-scented geranium (*Pelargonium* species) essential oil. *Bioresource Technology*, **84**, 243–246.
- Marsh, D.F. (1948) The comparative pharmacology of the isomeric hyptylamines. *Journal of Pharmacology and Experimental Therapeutics*, **94**, 225–231.
- Swanson, E.E., Chen, K.K. (1948) Comparison of pressor action of alicyclic derivatives of aliphatic amines. *Journal of Pharmacology and Experimental Therapeutics*, **93**, 423–429.
- Shonle, H.A., Rohrmann, E.U.S. Patent Office, Patent No: 2,350,318; (1944).
- Gee, P., Jackson, S., Easton, J. (2010) Another bitter pill: A case of toxicity from DMAA party pills. *New Zealand Medical Journal*, **123**, 124–127.
- Classification of 1,3-Dimethylamylamine (DMAA), Health Products and Food Branch. *Health Canada*, (2011).
- Govt moves to restrict party pill drug, TVNZ News, November 07, 2009. <http://tvnz.co.nz/health-news/govt-moves-restrict-party-pill-drug-3117890> (accessed February 2012).
- The 2010 Prohibited List—International Standard, The World Anti-Doping Agency, 2010. <http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/> (accessed February 2012).
- Unnamed Australians test positive for banned substance, BBC Sport, October 23, 2010. http://news.bbc.co.uk/sport2/hi/front_page/9120842.stm (accessed February 2012).
- Rui Costa and his brother test positive, Cycling News, October 19, 2010. <http://www.cyclingnews.com/news/rui-costa-and-his-brother-test-positive> (accessed February 2012).
- Kendrick banned until May after taking jetlag tablet before French Open, Mail Online UK, July 30, 2011. <http://www.dailymail.co.uk/sport/tennis/article-2020445/Robert-Kendrick-banned-taking-jetlag-tablet.html> (accessed February 2012).
- Premarket Notification for a New Dietary Ingredient. 62 FR 49886 (1997). Department of Health and Human Services. <http://www.fda.gov/Food/DietarySupplements/GuidanceComplianceRegulatoryInformation/ucm107929.htm> (accessed February 2012).
- Company, O.H.-W.D. (1955) A sales builder that sells on sight (advertisement). *The Carolina Journal of Pharmacy*. North Carolina Pharmaceutical Association at Chapel Hill, NC.
- Federal Register / Vol. 48, No. 218 / Wednesday, November 9, 1983 / Notices. (1983).
- Salinger, L., Daniels, B., Sangalli, B., Bayer, M. (2011) Recreational use of a body-building supplement resulting in severe cardiotoxicity. *Clinical Toxicology (Phila)*, Please provide the date this website was accessed for the study., 573–574.
- Bloomer, R.J., Harvey, I.C., Farney, T.M., Bell, Z.W., Canale, R.E. (2011) Effects of 1,3-dimethylamylamine and caffeine alone or in combination on heart rate and blood pressure in healthy men and women. *Physician and Sportsmedicine*, **39**, 111–120.
- Tittern, T.J. (2011) Army probing connection between body building supplement, 2 deaths. *Stars and Stripes*. <http://www.stripes.com/news/army-probing-connection-between-body-building-supplement-2-deaths-1.163652> (accessed February 2012).