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

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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.

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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]

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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.

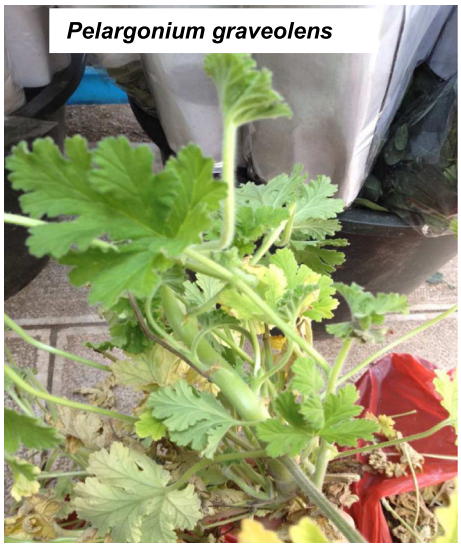

Figure 1. ***Pelargonium graveolens:*** The plant material was provided by Dr. Yi Jin at the School of Chemical Science and Technology Yunnan University, Kunming, Yunnan, China. The authentication was performed by Herbarium, Kunming Institute of Botany Chinese Academy of Science, Kunming, Yunnan, China.

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.

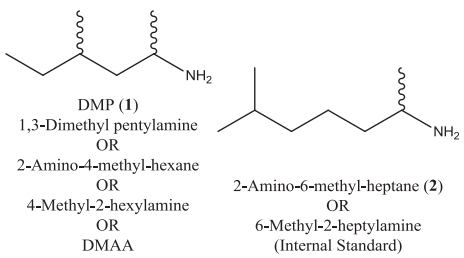

Figure 4. Chemical structures of MHA and the internal standard used for the GC-MS analysis.[1]

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

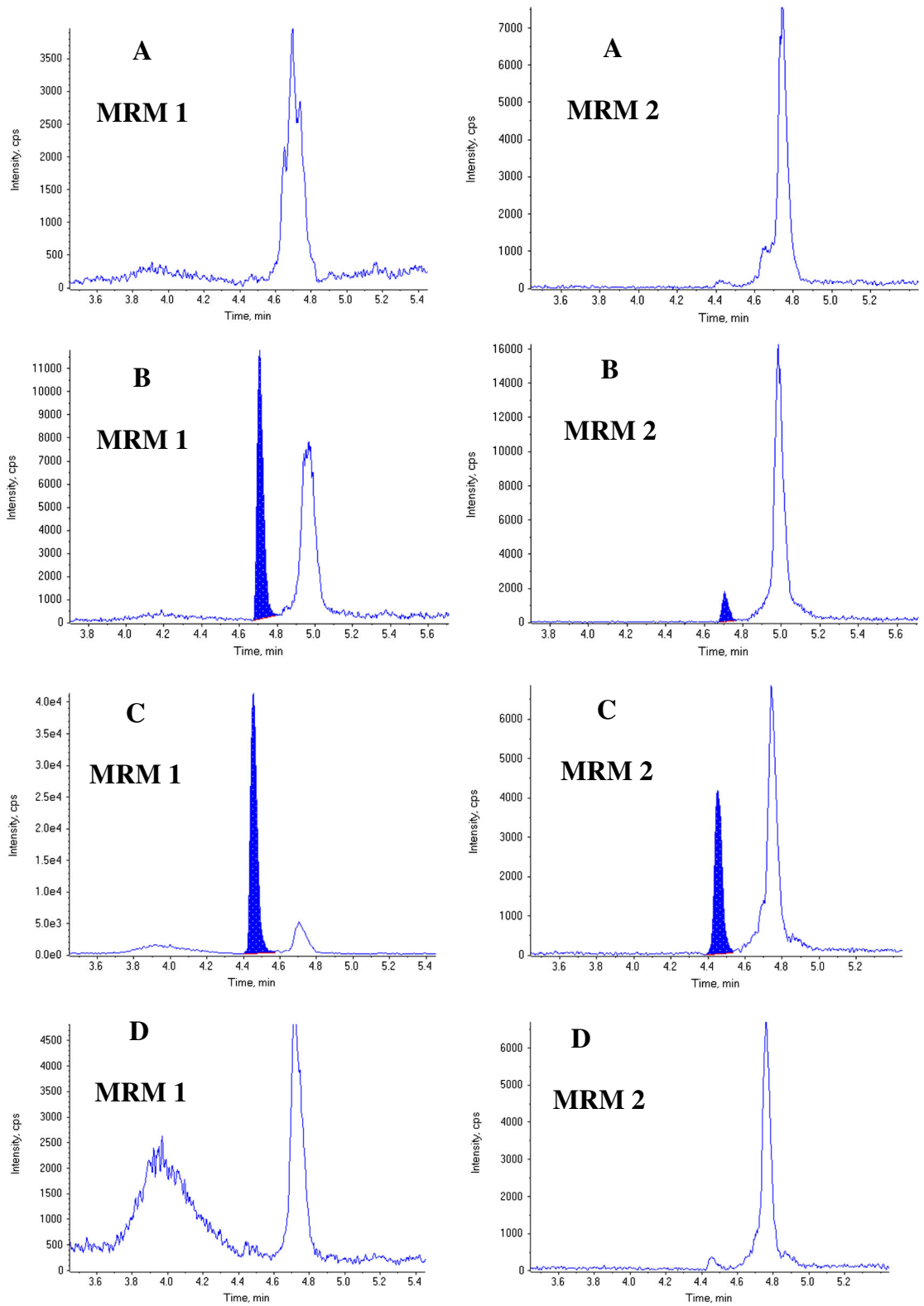

Figure 5. Chromatograms showing MRM-1 (figures on the left) and MRM-2 (figures on the right) of MHA for an unspiked sample of *Pelargonium graveolens* L'Her from Yunnan Province (**A** chromatograms), with no peaks at R_t of MHA (4.47 min) and those of the same sample spiked at 10 ng/mL MHA (showing 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

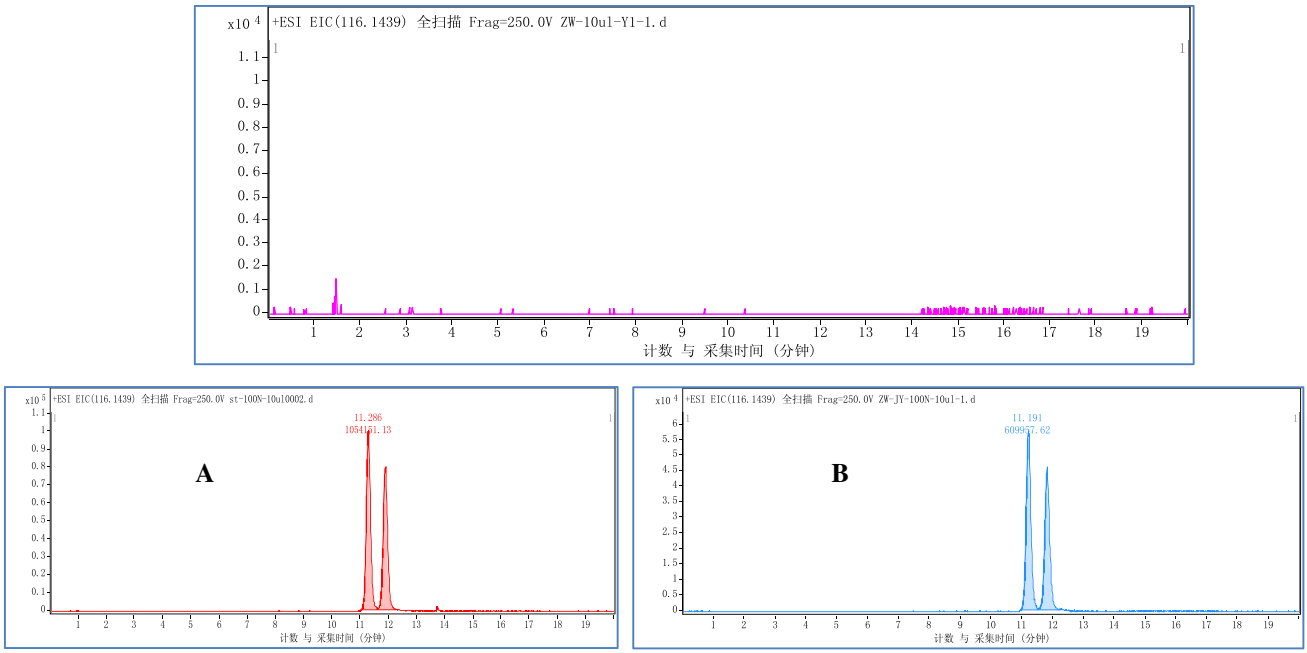

Figure 6. EIC of the extract of Plant 1 (top figure) and those of a MHA standard (bottom left, **A**) and the extract of a plant sample spiked with 1 μg of MHA (bottom right, **B**) (School of Pharmacy, Second Military Medical University Shanghai China).

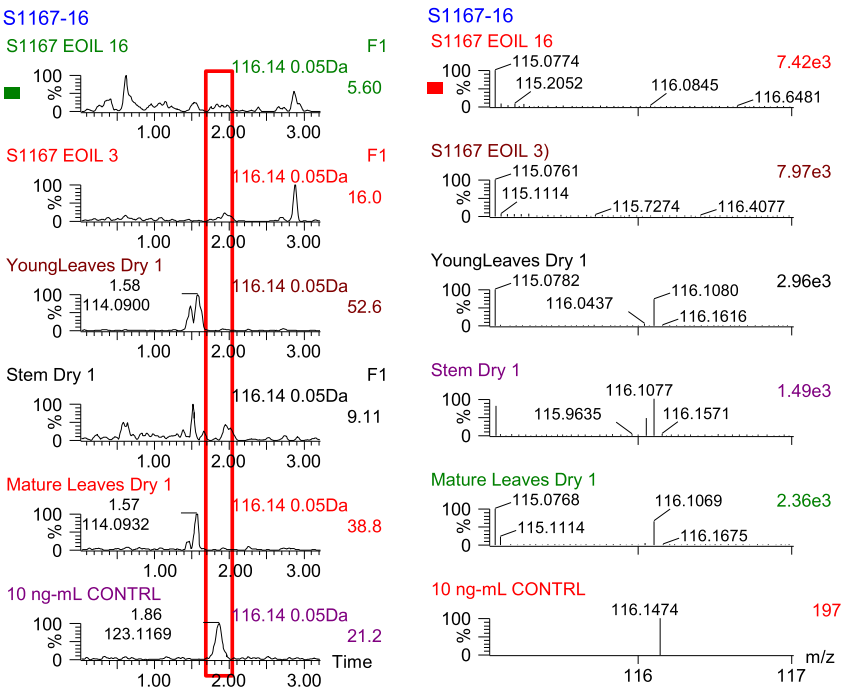

Figure 7. LC-MS-QTOF chromatograms of plant materials, oil samples and a 10 ng/mL control and the full scan accurate masses for the peaks at R_t of MHA. Note: The peak was around 1.84 min and the mass was found to be 116.10 for all samples analyzed [(where the MHA mass should be 116.14 (accurate mass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

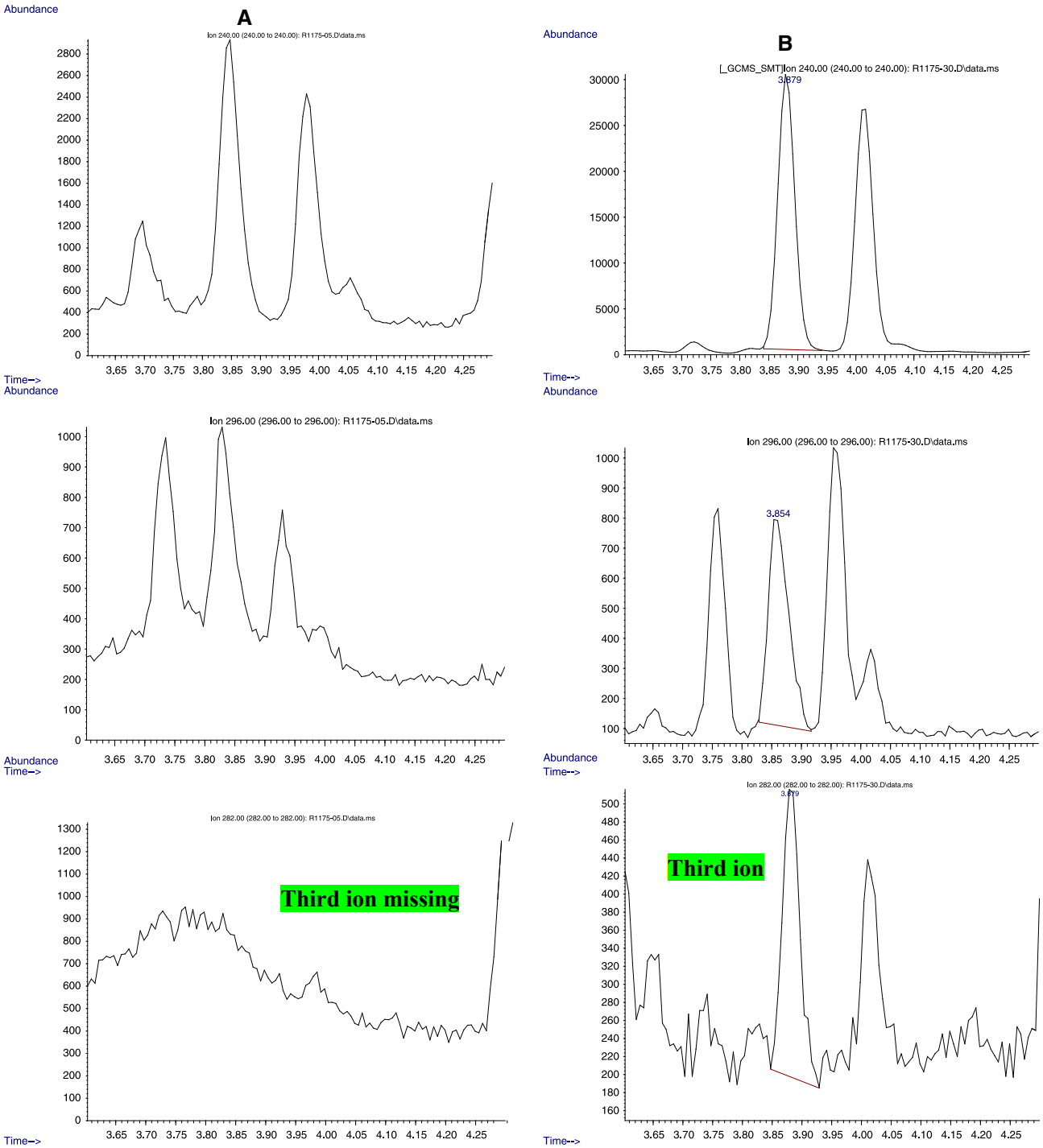

Figure 8. **(A)** GC-MS selected ion chromatograms of ions at *m/z* 240, 296, and 282 of the MHA-HFB derivative of a negative geranium oil control; and (**B**) GC-MS selected ion chromatograms of the same ions for a negative geranium oil spiked at 0.1 ppm MHA. Note the absence of ions at *m/z* 282 in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

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