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Validated RP-UHPLC PDA and GC–MS methods for the analysis of psychoactive alkaloids in *Sceletium tortuosum*

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Abstract

Anecdotal evidence indicates that *Sceletium tortuosum* has been used as a psychoactive preparation for several centuries. The psychological activity is attributed to the production of mesembrine-type alkaloids (mesembrenol, mesembranol, mesembrenone and mesembrine) by the plant. This investigation was aimed at developing validated reversed phase ultra performance liquid chromatography with photodiode array detector (RP-UHPLC PDA) and gas chromatography coupled to mass spectrometry (GC–MS) methods for quantitative assessment of mesembrine-type alkaloids in *S. tortuosum* raw materials and products. Both methods were validated for linearity, repeatability and recovery. Limits of detection (LOD) and limits of quantification (LOQ) for the alkaloids were also established. The LOD range for the RP-UHPLC PDA analysis of compounds was 0.91–1.21 μg ml^{-1}, while the LOQ range was 2.72–3.71 μg ml^{-1}. For GC–MS analysis, the LOD and LOQ ranges for the compounds were 11.78–22.76 μg ml^{-1} and 35.34–68.29 μg ml^{-1} respectively. Regression analyses indicated good linear relationships for the investigated compounds. The regression coefficients (R^2) from RP-UHPLC PDA analyses ranged from 0.9977 to 0.9991, while those derived from GC–MS analyses were within the range of 0.9979 to 0.9995. Intra- and inter-day precision values for all the alkaloids were below 3.44% for RP-UHPLC PDA and below 3.15% for GC–MS analysis, indicating excellent repeatability. Percentage recovery ranges for the four alkaloids as determined by RP-UHPLC PDA and GC–MS analyses ranged from 80.00 to 89.83% and from 80.77 to 90.00%, respectively. Plots of RP-UHPLC PDA data of selected raw materials and products versus GC–MS data, showed good linear correlation (R^2 = 0.9820–0.9987). The chromatographic methods were found to be repeatable, precise and appropriate for use in the routine quality control of the psychoactive alkaloids in *S. tortuosum* raw materials and products.

Keywords: GC–MS; Mesembrine; Method validation; Precision; RP-UHPLC; *Sceletium tortuosum*

1. Introduction

Depression and stress, though typically not regarded as diseases, are recurrent life threatening heterogeneous disorders associated with diverse behavioural, psychological and physiological symptoms (Minkoff et al., 1973). Reports have indicated that approximately 30% of patients afflicted with these conditions do not respond to conventional medical treatment (Lickerman, 2010). Plants that produce molecules exhibiting antidepressant and immune-modulation properties have therefore become attractive targets for the development of new drugs for treatment of central nervous system (CNS) disorders (Kumar, 2006).

Sceletium tortuosum (L.) N.E.Br (Mesembryanthemaceae) has also been targeted due to its application in the treatment of neurological disorders and neurodegenerative diseases (Van Wyk and Gericke, 2003). The species, indigenous to South Africa, is a decumbent perennial sub-shrub that forms part of the dry Karoo vegetation of the south-western regions of the country. The plant is commonly referred to as "*kanna*" *or* "*kougoed*" by local communities and is traditionally used to treat CNS-related conditions (Van Wyk and Gericke, 2003). In addition, it is used in psychoactive preparations for quenching thirst, for relief of hunger and as a colic remedy for babies (Smith et al., 1996; Van Wyk and Gericke, 2003). Phytochemical studies of *S. tortuosum* have

Abbreviations: CNS, central nervous system; ICH, International Conference of Harmonisation; PDE, phosphodiesterase; SIM, selective ion monitoring; PDA, photodiode array; S_a, uncertainty in the y intercept; S_b, uncertainty in the slope; $S_{y/x}$, random calibration uncertainty.

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shown that alkaloids, commonly referred to as "mesembrine-type alkaloids" (mesembrine, mesembrenone, mesembrenol and mesembranol) are responsible for the psychoactive properties of the plant (Smith et al., 1996; Van Wyk and Gericke, 2003). The ability of these alkaloids to treat CNS-related disorders has been associated with their role in serotonin re-uptake inhibition and phosphodiesterase 4 (PDE4) inhibition (Gericke and Viljoen, 2008; Patnala and Kanfer, 2009).

Owing to the chemical diversity and varying abundance of plant metabolites in phytomedicines, it is difficult to employ a single procedure for analysis of all metabolites in a particular medicinal plant (Li et al., 2007). In addition to other analytical techniques, chromatographic methods such as TLC, HPLC, UHPLC and GC have played a crucial role in elucidating the phytochemistry of medicinal plants for quality assurance purposes. It is well known that specified markers or pharmacologically active compounds are generally used as standards for the quality control of traditional medicines (Li et al., 2007). On the basis of the available data, GC–MS, RP-HPLC and TLC have been used for qualitative analyses of alkaloids in *Sceletium* (Gericke and Van Wyk, 1999; Patnala and Kanfer, 2010; Smith et al., 1998). However, only HPLC with photodiode array (PDA) detection has been reported for the quantitative determination of these alkaloids (Patnala and Kanfer, 2010). In recent years there has been a drastic increase in the number of companies marketing *Sceletium* products in various formulations (capsules, tablets, sprays, teas and tinctures) via the internet (www.Sceletium.org). However, reports on validated analytical methods for quantitative determination of the alkaloids in these products are scarce (Patnala and Kanfer, 2010). This investigation was therefore, aimed at developing validated standard analytical protocols for routine monitoring and quantification of the psychoactive alkaloids in *S. tortuosum* raw materials and products using RP-UHPLC PDA and GC–MS methods. The proposed methods were validated for linearity, precision and repeatability in accordance with the International Conference of Harmonisation (ICH) guidelines (ICH Q2(R1), 2005). In addition, the limits of detection (LOD) and quantification (LOQ) of the methods were established.

2. Materials and methods

2.1. Reagents and materials

All solvents used for extraction, including methanol (MeOH), dichloromethane (DCM), ammonia (NH_3; 25% w/w solution) and sulfuric acid (H_2SO_4; 98.08% w/w), were of analytical grade with the exception of HPLC grade acetonitrile, which was used for RP-UHPLC PDA analysis. The solvents were procured from Merck™ (Germany). Distilled water was filtered using hydrophilic 0.25 μm Millex-HV membranes from Millipore (Johannesburg, South Africa). *Sceletium* reference standards (mesembrenol, mesembranol, mesembrenone and mesembrine), were previously isolated from *S. tortuosum* by a combination of column chromatography and high speed countercurrent chromatography (Shikanga et al., 2011a). The identity and purity of each alkaloid were confirmed by TLC, HPLC, GC–MS and ^{1}H and ^{13}C (1 and 2D) NMR, as well as by comparing their spectral data with literature data.

2.2. Sample preparation

Aerial parts of wild *S. tortuosum* plants were collected from various localities in the south-western region of South Africa in October 2009 and voucher specimens were deposited in the Department of Pharmaceutical Sciences, Tshwane University of Technology. Plant material with voucher specimen numbers SCT019 collected from Oudsthoorn and, SCT077, SCT082 and SCT103, from Steildrift, was selected for the analyses. Commercial capsules (Com1, 4, 7 and 8), containing *S. tortuosum* powder were purchased from health shops and local manufacturers in South Africa. The extraction method was optimised as described previously (Shikanga et al., 2011b), by comparing the extraction efficiencies of two solvents (water and MeOH) to that of an acid-base method reported by Alali et al. (2008). The last was found to be the most effective for the extraction of alkaloids from *S. tortuosum* plant material.

Leaves of the various plant specimens were dried in an oven (Labotec Ltd) at 30 °C for two weeks prior to extraction. A Retsch® MM 400 ball mill (Monitoring and Control (Pty) Ltd; Haans, Germany) was used to pulverise the dry material to fine powder at a frequency of 30 Hz for 2 min. The pulverised plant material was then sieved using a 500 μm mesh (Endcotts Filters Ltd; London, UK). Alkaloids were extracted from the sieved powder using the conventional acid-base extraction method for alkaloid extraction (Alali et al., 2008), with slight modifications. A volume of 24.0 ml 0.5 M H_2SO_4 was added to 2.0 g of each sample in a 25 ml centrifuge tube and the mixture was vortexed (Vortex Gene vortex; Scientific Industries™, Florida, USA) at 200 rpm for 15 s. The tubes were then centrifuged using a Jouan BR4i centrifuge (DJB Lab Care®; Buckinghamshire, UK) at 5000 rpm for 15 min, before filtering the supernatant from each tube into a fresh 25 ml tube. The resulting filtrates were each neutralised using 6.0 ml of 20% aqueous ammonia.

A volume of 14.0 ml of DCM was then added to the basic content of each tube, which was swirled manually for 15 s. The mixtures were left to settle and the organic (DCM) phase in each tube was filtered into a clean 25 ml glass vial. Extraction with DCM was repeated and the two resulting filtrates were combined and dried in a Vismara vacuum oven (Vismara Srl Scientific™; Milan, Italy) at 40 °C and 0.2 bar. A 2.0 g mass of powder, removed from the capsules of each of the commercial products, was extracted in the same way as described for the wild samples.

2.3. Instrumentation

Chromatographic profiling of *S. tortuosum* extracts (wild and commercial products) and quantification of the four alkaloids were done using RP-UHPLC PDA and GC–MS systems. The UHPLC system was comprised of a Waters Acquity ultra performance liquid chromatographic sample manager (Waters™; Ireland), a binary solvent manager and a PDA detector (210–400 nm). Separation was achieved using a Waters Acquity reversed phase

UHPLC BEH C18 (2.1×150 mm, 1.7 μm particle size) column and a Van Guard pre-column (2.1×5 mm, 1.7 μm).

Analysis by GC–MS was conducted using an Agilent 6890N gas chromatograph, coupled to a 5973 mass spectrometer. Sample separation was achieved using a HP-5MS 5% phenyl methyl siloxane column (30 m×250 μm i.d.×0.25 μm film thickness).

2.4. Method development

Masses of 5.1 mg of mesembrine (purity: 98.5%), mesembrenol (98.4%), mesembrenone (98.2%), and 5.2 mg of mesembranol (95.4%) were each separately weighed and quantitatively transferred into a 5.0 ml volumetric flask with MeOH. The mixture was dissolved by sonication in a Sonorex digital 10P sonic bath (Bandelin Electronic™; Berlin, Germany) for 5 min at 25 °C, before adjusting the volume to the graduation mark. This stock solution, containing each of the four alkaloids at a concentration of 1.0 mg ml^{-1}, was appropriately diluted to prepare six calibration standards for each of the analytical methods. The concentrations of the calibration standards for RP-UHPLC PDA ranged from 5.00 to 100.00 μg ml^{-1}, while those for GC–MS analysis ranged from 0.10 to 1.00 mg ml^{-1} for each of the four alkaloids. Sample extracts, prepared as described in Section 2.2, were diluted to 1.0 mg ml^{-1} and 5.0 mg ml^{-1} in MeOH for RP-UHPLC PDA and GC–MS analyses, respectively.

An injection volume of 1 μl was used for RP-UHPLC PDA analysis. Sample and column temperatures were adjusted to 25 and 30 °C, respectively. The mobile phase (0.3 ml min^{-1}) consisted of (A) 0.1% aqueous ammonia and (B) MeOH. Gradient elution was employed, starting with 80% A and 20% B, changing to 40% B in 2 min, then changing to 50% B in 2 min and held constant for 3 min, with a post-run time of 1 min. A wavelength of 280 nm (resolution 1.2 nm) was selected as the most appropriate for the analysis.

For GC–MS analysis, splitless injection (2 μl) was done using an auto-injector and an auto-sampler at 24.0 psi and an inlet temperature of 250 °C. The oven temperature programme was set at an initial temperature of 259 °C, held constant for 2 min, and raised to 262 °C at a rate of 20 °C min^{-1} and then held constant for 6 min. Carrier gas (helium) flow rate was maintained at 1.4 ml min^{-1} and spectra were obtained following electron impact ionization at 70 eV (35 to 550 *m*/*z*). The transfer line temperature was set at 280 °C. Alkaloids were identified in the extracts after comparison of their retention times and MS fragmentation patterns with those of authentic standards and spectral data in the NIST® Library version 2004.

2.5. Method validation

Quantitative determinations of the four alkaloids (mesembrenol, mesembranol, mesembrenone and mesembrine) in *S. tortuosum* raw materials and products, using RP-UHPLC PDA and GC–MS methods were validated for linearity, recovery and repeatability in accordance with the ICH Guidelines (ICH Q2(R1), 2005).

2.5.1. Linearity

A six-point calibration curve was prepared for each analyte, from RP-UHPLC PDA and GC–MS analyses. The linearity of each curve, representing a specific alkaloid, was determined by regression analysis (Microsoft Excel 2007).

2.5.2. Limits of detection and quantification

Determination of the LOD and LOQ is important when evaluating the active constituents in natural products such as botanical samples, since the quantities of these components may be highly variable (Renger et al., 2011). The LOD and LOQ for each alkaloid and for both analytical methods were calculated following regression analyses of the calibration data (Miller and Miller, 2000; ICH Q2(R1), 2005).

2.5.3. Repeatability

Repeatability (intra- and inter-day assays) of each compound was determined by independently extracting and analysing a real sample (Sample SCT077) repeatedly. For intra-day assay, 0.50, 1.00 and 1.50 g of pulverised plant material were extracted in triplicate (Section 2.2) and analysed using RP-UHPLC PDA and GC–MS methods at three different times within a day. Each extract was diluted to 2.0 and 10.0 mg ml^{-1} in MeOH before analysis using RP-UHPLC PDA and GC–MS, respectively. The results obtained were expressed as percentage relative standard deviation (%RSD). This procedure was repeated for an additional two days in order to determine inter-day repeatability ($n=9$).

2.5.4. Recovery

The recovery of each analyte was determined by means of a standard addition assay. Standards were added to the sample at three quantitative levels representing low, medium and high spikes for each analyte. Pulverised material of Sample SCT077 was weighed (1.00 g) into four separate 25 ml centrifuge tubes. No standard was added to the sample in the first tube, while 0.50, 1.00 and 1.50 mg of each alkaloid were added to the sample in the second, third and fourth tubes, respectively. All of the samples were then extracted as described in Section 2.2. Aliquots of the extracts were diluted to 10.00 mg ml^{-1} for GC–MS analysis and further diluted to 4.00 mg ml^{-1} for RP-UHPLC PDA analysis with MeOH. The recovery of each alkaloid was expressed as the percentage concentration of the compound recovered against the concentration of the relevant spike.

2.6. Analysis of mesembrine-type alkaloids in raw materials and products

The dry alkaloid-enriched extracts, resulting from acid-base extraction, were each reconstituted in MeOH at a concentration of 10.00 mg ml^{-1} prior to RP-UHPLC PDA and GC–MS analyses. Both validated methods were used to quantify the four alkaloids in the selected raw materials and products.

2.7. Data analysis

Statistical analyses, including regression analysis, calculation of means, standard deviation (SD), relative standard deviation

(%RSD) and one-way analysis of variance (ANOVA, single factor without replication) were performed using Microsoft Excel 2007. Data obtained from RP-UHPLC PDA and GC–MS analyses were compared by regression analysis. A one-way analysis of variance (ANOVA, single factor without replication) was also used to compare data obtained from RP-UHPLC PDA analysis to that from GC–MS analysis. Values with $p \geq 0.05$ are not significantly different.

3. Results and discussion

Satisfactory resolution of the alkaloids, with no peak overlap evident, was achieved by both analytical methods within 8.0 min. Compounds were quantified, based on peak area calibration for the RP-UHPLC PDA method and on selective ion monitoring (SIM) for the GC–MS method. Quantification by SIM was performed by targeting a set of three fragment ions for each alkaloid. Mass spectra and structures of the four alkaloids are shown in Fig. 1. The *m/z* values of the ions selected for the quantification of the four alkaloids were 204, 274 and 290 for mesembrenol, 70, 232 and 289 for mesembranol, 219, 258 and 287 for mesembrenone and 96, 218 and 289 for mesembrine. Linearity of an analytical method has been expressed as the ability to obtain measurements that are directly proportional to the concentrations of an analyte within a given range (ICH Q2(R1), 2005; Rajpur and Sonanis, 2011). In this study, linear relationships were obtained within the calibration ranges selected for each analyte as reflected by the regression coefficients (R^2) (0.9984 to 0.9991 for RP-UHPLC PDA and 0.9979 to 0.9995 for GC–MS). The regression results derived from calibration curves for each analyte, obtained from data collected within three days, are illustrated in Tables 1 and 2 for the RP-UHPLC PDA and GC–MS methods, respectively.

Values representing the uncertainties in the y intercept (S_a), slope (S_b) and the random calibration uncertainty ($S_{y/x}$) of each calibration were determined with a 95% confidence limit. The S_a values were found to be greater than S_b values in all analyses, indicating that an adequate range was selected for the calibration of each analyte (Miller and Miller, 2000). The chosen concentrations of the standards were suitable, since the S_a and S_b values obtained were lower than those of $S_{y/x}$ for each calibration. Moreover, the F_{calc} values were greater than the corresponding F_{sig} values in all the calibrations, indicating significant linearity. The LOD and LOQ for each alkaloid from RP-UHPLC PDA and GC–MS analyses (Tables 1 and 2, respectively) reflected the significantly greater sensitivity of the RP-UHPLC

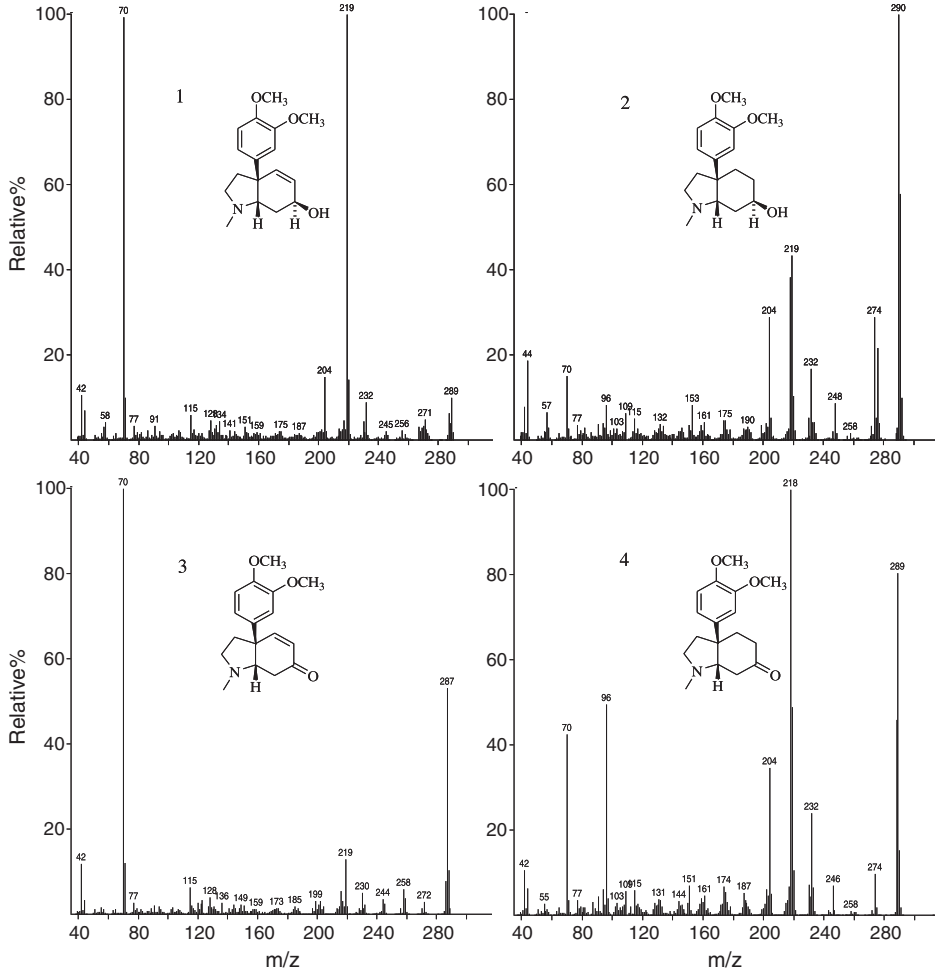

Fig. 1. Chemical structures of mesembrine-type alkaloids and their mass spectra from GC–MS analysis. 1) Mesembrenol; 2) mesembranol; 3) mesembrenone; 4) mesembrine.

Table 1
Regression results, LOD and LOQ values from RP-UHPLC PDA analysis of alkaloids from *Sceletium tortuosum*.

Analyte	Rt [a] (min)	Regression equation (10^3)	Regression coefficient (R^2)	S_a [b] ($\times 10^3$)	S_b [c]	$S_{y/x}$ [d] ($\times 10^3$)	F_{calc} ($\times 10^3$)	F_{sig} ($\times 10^{-7}$)	LOD [e] ($\mu g\ ml^{-1}$)	LOQ [f] ($\mu g\ ml^{-1}$)
Mesembrenol	3.43	y=3.26x−0.63	0.9987	0.92	19.55	0.99	3.15	6.02	0.91	2.72
Mesembranol	3.68	y=3.29x−1.01	0.9991	1.08	22.92	1.19	4.26	3.3	1.04	3.25
Mesembrenone	3.90	y=6.98x−1.50	0.9977	2.55	75.38	2.72	1.76	19.41	1.15	3.60
Mesembrine	4.64	y=3.20x−1.22	0.9984	1.04	22.13	1.35	2.51	9.52	1.21	3.71

95% confidence limit.
[a] Rt = retention time.
[b] S_a = uncertainty in the y intercept.
[c] S_b = uncertainty in slope.
[d] $S_{y/x}$ = random calibration uncertainty.
[e] LOD = limit of detection.
[f] LOQ = limit of quantification.

Table 2
Regression results, LOD and LOQ values from GC–MS analysis of alkaloids from *Sceletium tortuosum*.

Analyte	Rt [a] (min)	Regression equation ($\times 10^4$)	Regression coefficient (R^2)	S_a [b] ($\times 10^3$)	S_b [c] ($\times 10^3$)	$S_{y/x}$ [d] ($\times 10^3$)	F_{calc} ($\times 10^3$)	F_{sig} ($\times 10^{-7}$)	LOD [e] ($\mu g\ ml^{-1}$)	LOQ [f] ($\mu g\ ml^{-1}$)
Mesembrenol	3.56	y=53.12x−5.92	0.9988	9.12	5.99	1.10	3.25	5.74	11.78	35.34
Mesembranol	3.59	y=812.23x−160.15	0.9995	9.53	6.39	11.21	4.81	2.60	13.04	39.12
Mesembrenone	4.16	y=724.12x−39.21	0.9981	10.57	8.24	11.66	155.84	14.15	19.02	57.07
Mesembrine	4.48	y=663.36x−42.00	0.9979	10.79	11.41	12.84	146.59	18.32	22.76	68.29

95% confidence limit.
[a] Rt = retention time.
[b] S_a = uncertainty in the y intercept.
[c] S_b = uncertainty in slope.
[d] $S_{y/x}$ = random calibration uncertainty.
[e] LOD = limit of detection.
[f] LOQ = limit of quantification.

PDA method (LOD: 0.91–1.21; LOQ: 2.72–3.71 $\mu g\ ml^{-1}$) as compared to the GC–MS method (LOD: 11.78–22.76; LOQ: 35.34–68.29 $\mu g\ ml^{-1}$).

Results for intra- and inter-day repeatability analyses of sample SCT077 using RP-UHPLC PDA and GC–MS methods are summarized in Tables 3 and 4, respectively. The intra-day precision data are presented as %RSD values, calculated for each alkaloid in the sample. These values were found to be below 3.00% for both RP-UHPLC PDA and GC–MS analyses, indicating that the analysis performed at different times

Table 3
Intra- and inter-day RP-UHPLC PDA repeatability study for mesembrine-type alkaloids from *Sceletium tortuosum*.

Analyte	Analyte level	Intra-day analysis						Inter-day analysis	
		Day 1		Day 2		Day 3			
		Observed conc. [a] ($\mu g\ ml^{-1}$)	%RSD	Observed conc. [a] ($\mu g\ ml^{-1}$)	%RSD	Observed conc. [a] ($\mu g\ ml^{-1}$)	%RSD	Observed conc. [b] ($\mu g\ ml^{-1}$)	%RSD
Mesembrenol	Low	21.37±0.45	2.11	21.17±0.45	2.13	21.23±0.49	2.32	21.20±0.52	2.44
	Medium	41.20±0.70	1.70	40.87±0.65	1.59	40.60±0.66	1.62	40.98±0.69	1.69
	High	67.43±0.76	1.13	67.50±0.66	0.97	67.67±0.64	0.95	67.38±0.80	1.19
Mesembranol	Low	10.10±0.26	2.62	10.07±0.25	2.50	10.00±0.30	3.00	10.07±0.35	3.44
	Medium	19.07±0.31	1.60	18.87±0.25	1.33	18.77±0.25	1.34	18.84±0.34	1.78
	High	32.90±0.47	1.22	32.73±0.45	1.38	32.93±0.45	1.37	32.77±0.49	1.51
Mesembrine	Low	5.37±0.15	2.85	5.27±0.15	2.90	5.17±0.15	2.96	5.27±0.16	3.00
	Medium	10.57±0.35	3.32	10.20±0.30	2.94	10.10±0.30	2.97	10.29±0.35	3.38
	High	18.20±0.46	2.52	18.27±0.35	1.92	18.20±0.26	1.45	18.26±0.44	2.41
Mesembrenone	Low	14.17±0.25	1.78	14.03±0.25	1.79	14.06±0.20	1.43	14.03±0.28	1.98
	Medium	26.80±0.56	2.08	26.77±0.35	1.31	26.97±0.50	1.87	26.87±0.57	2.13
	High	45.27±0.76	1.69	45.83±0.74	1.61	45.87±0.45	0.98	45.69±0.76	1.65

[a] Each observed conc value for intra-day repeatability = mean ± SD (n=3).
[b] Each observed conc value for inter-day repeatability = mean ± SD (n=9).

Table 4
Intra- and inter-day GC–MS repeatability study for mesembrine-type alkaloids from *Sceletium tortuosum*.

Analyte	Analyte level	Intra-day analysis						Inter-day analysis	
		Day 1		Day 2		Day 3			
		Observed conc. [a] (μg ml^{-1})	%RSD	Observed conc. [a] (μg ml^{-1})	%RSD	Observed conc. [a] (μg ml^{-1})	%RSD	Observed conc. [b] (μg ml^{-1})	%RSD
Mesembrenol	Low	427.13±9.02	2.11	423.98±9.02	2.13	423.61±8.14	1.92	424.78±9.32	2.20
	Medium	824.00±14.00	1.70	817.34±13.01	1.59	812.4±13.11	1.62	815.89±13.44	1.65
	High	1348.47±5.28	1.13	1350.71±13.11	0.97	1353.12±12.86	0.95	1346.00±15.84	1.18
Mesembranol	Low	202.80±5.29	2.62	201.31±5.03	2.50	200.21±6.00	3.00	200.22±6.04	3.02
	Medium	381.38±6.11	1.60	377.13±5.03	1.33	375.32±5.03	1.34	376.56±6.35	1.69
	High	658.09±8.00	1.22	654.68±9.02	1.38	658.62±9.02	1.37	653.78±9.35	1.43
Mesembrine	Low	107.41±3.06	2.85	105.35±3.06	2.90	103.72±3.06	2.96	280.00±5.20	1.86
	Medium	211.35±7.01	2.81	204.98±6.00	2.94	202.06±6.00	2.97	535.67±11.00	2.05
	High	362.67±7.02	1.94	365.89±7.02	1.92	364.01±5.29	1.45	916.11±15.30	1.67
Mesembrenone	Low	283.43±5.03	1.78	280.69±5.03	1.79	280.22±4.00	1.43	105.33±3.16	3.00
	Medium	536.06±11.14	2.08	535.33±7.02	1.31	539.33±10.07	1.87	206.11±6.49	3.15
	High	905.35±15.28	1.69	916.67±14.74	1.61	917.33±9.02	0.98	362.11±7.42	2.05

[a] Each observed conc value for intra-day repeatability = mean ± SD (n = 3).
[b] Each observed conc value for inter-day repeatability = mean ± SD (n = 9).

throughout the day yielded precise results. Data calculated for the inter-day precision of each compound by both methods were determined by comparing nine measurements made during analyses conducted over three days. The results of these analyses (Tables 3 and 4 for RP-UHPLC PDA and GC–MS methods, respectively) indicated excellent repeatability over three days with %RSD values for the alkaloids ranging from 1.19 to 3.44% for RP-UHPLC PDA and from 1.18 to 3.15% for GC–MS analysis. Values representing the recovery data from RP-UHPLC PDA and GC–MS analyses of Sample SCT077, spiked with four reference standards, are presented in Tables 5 and 6. The recoveries of the four alkaloids were found to be in the ranges of 80.00–89.83% (RP-UHPLC PDA analysis) and 80.77–90.00% (GC–MS analysis). All these recovery values were within the range of 80 to 120%, which is regarded as acceptable for compounds in botanical substances according to published reports (ICH Q2(R1), 2005; Renger et al., 2011).

The validated RP-UHPLC PDA and GC–MS methods were used for the quantification of the four alkaloids in the same selected wild samples and commercial products of *S. tortuosum*. Sample RP-UHPLC PDA chromatograms of a raw material (SCT077) and a product (Com1) are depicted in Fig. 2. Quantitative data of the alkaloids in the raw materials and commercial products obtained from RP-UHPLC PDA analysis were compared to those obtained from GC–MS analysis by constructing plots of the RP-UHPLC PDA values as a function of their respective GC–MS values for each alkaloid (Fig. 3A–D). The regression coefficient values obtained for each of the four alkaloids indicated linear relationships with R^2 values ranging from 0.9820 to 0.9987. A linear relationship proves that data obtained from both analytical methods are similar.

Quantitative data for the four alkaloids in the samples as determined by the two methods are presented in Fig. 4. Qualitative and quantitative variations of the alkaloids were evident both in the raw materials and products investigated in the study. The four

Table 5
Results of recovery studies for mesembrine-type alkaloids from *Sceletium tortuosum* (Sample SCT077) as determined by RP-UHPLC PDA analysis.

Analyte	Sample content (μg ml^{-1})	Spike (μg ml^{-1})	Sample + spiked content (μg ml^{-1})	Recovery content (μg ml^{-1})	%Recovery
Mesembrenol	53.44±1.15	5.00	57.43±1.16	4.03±0.26	80.67
		10.00	61.87±1.22	8.47±0.32	84.67
		20.00	70.67±2.76	17.27±0.46	86.33
Mesembranol	25.23±0.68	5.00	29.30±1.75	4.07±0.15	81.33
		10.00	33.67±0.86	8.43±0.21	84.33
		20.00	42.07±1.23	16.83±1.12	84.17
Mesembrenone	35.42±0.66	5.00	39.43±0.75	4.01±0.10	80.00
		10.00	44.09±2.82	8.62±0.30	86.00
		20.00	53.37±1.95	17.98±0.62	89.83
Mesembrine	13.47±0.30	5.00	17.47±0.55	4.07±0.23	81.33
		10.00	21.43±1.55	8.03±0.35	80.33
		20.00	30.33±2.15	16.93±0.45	84.67

Each value represents mean ± SD (n = 4).

Table 6
Results of recovery studies for mesembrine-type alkaloids from *Sceletium tortuosum* (Sample SCT077) as determined by GC–MS analysis.

Analyte	Sample content ($\mu g\ ml^{-1}$)	Spike ($\mu g\ ml^{-1}$)	Sample+spiked content ($\mu g\ ml^{-1}$)	Recovery content ($\mu g\ ml^{-1}$)	%Recovery
Mesembrenol	529.48±9.50	50.00	570.02±11.14	40.43±2.52	80.77
		100.00	614.25±11.59	84.77±2.31	84.26
		200.00	703.39±13.11	173.09±3.79	86.67
Mesembranol	253.55±5.13	50.00	294.36±4.04	40.67±1.15	81.38
		100.00	335.31±4.00	81.31±1.53	81.53
		200.00	423.22±8.54	169.09±11.55	84.54
Mesembrenone	351.69±9.29	50.00	392.65±8.33	41.05±3.00	82.12
		100.00	435.04±5.57	83.31±3.79	83.36
		200.00	529.67±9.87	178.00±4.73	90.00
Mesembrine	135.08±4.58	50.00	175.35±4.51	40.33±0.58	80.62
		100.00	218.00±5.29	83.05±2.65	83.04
		200.00	303.38±1.53	168.23±5.51	84.17

Each value represents mean±SD (n=4).

alkaloids were detected in all the samples and products investigated, with the exception of Com7, in which no mesembrenol was detected. Samples SCT077 and SCT082 contained higher levels of the alkaloids than SCT103, collected in the Steildrift area. Variations were also observed in the alkaloid profiles of these samples. Whereas mesembrenol was the most abundant alkaloid in Samples SCT077 and SCT103, mesembrenone was the main constituent of SCT082. This finding reveals variations in the alkaloid profiles of samples growing within the same locality. Differences in the relative amounts of alkaloids were also observed for sample growing in Oudtshoorn. Although mesembrine was found to be the most abundant compound in the commercial products, their alkaloid profiles were dissimilar, with the exception of Com1 and Com8. The high levels of mesembrine in the commercial products are possibly the result of companies using mesembrine levels as a standard to select plant material. This follows an early report by Van Wyk and Gericke (2003) indicating that mesembrine is the major active constituent of this species. However, recent in vitro experiments have shown that mesembrenol and mesembrenone also possess psychoactive properties (Harvey et al., 2011).

4. Conclusions

Simple, repeatable and precise RP-UHPLC PDA and GC–MS methods were developed for the identification and simultaneous

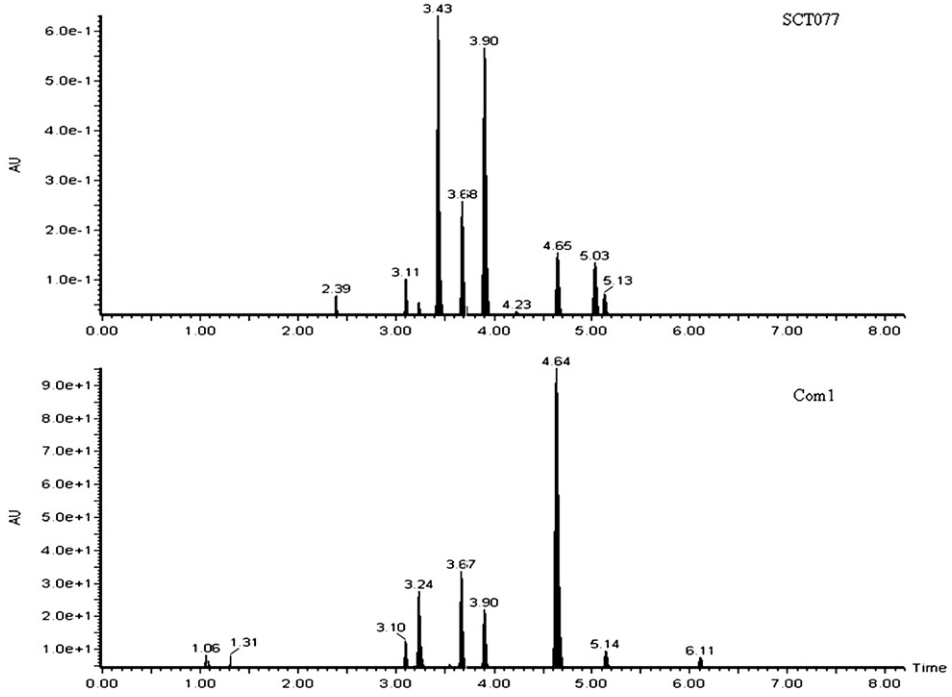

Fig. 2. Chromatograms of Sample SCT077 and Product Com1 as obtained by RP-UHPLC PDA analysis. Mesembrenol (Rt 3.43 min); mesembranol (Rt 3.68 min); mesembrenone (Rt 3.90 min); mesembrine (Rt 4.64 min).

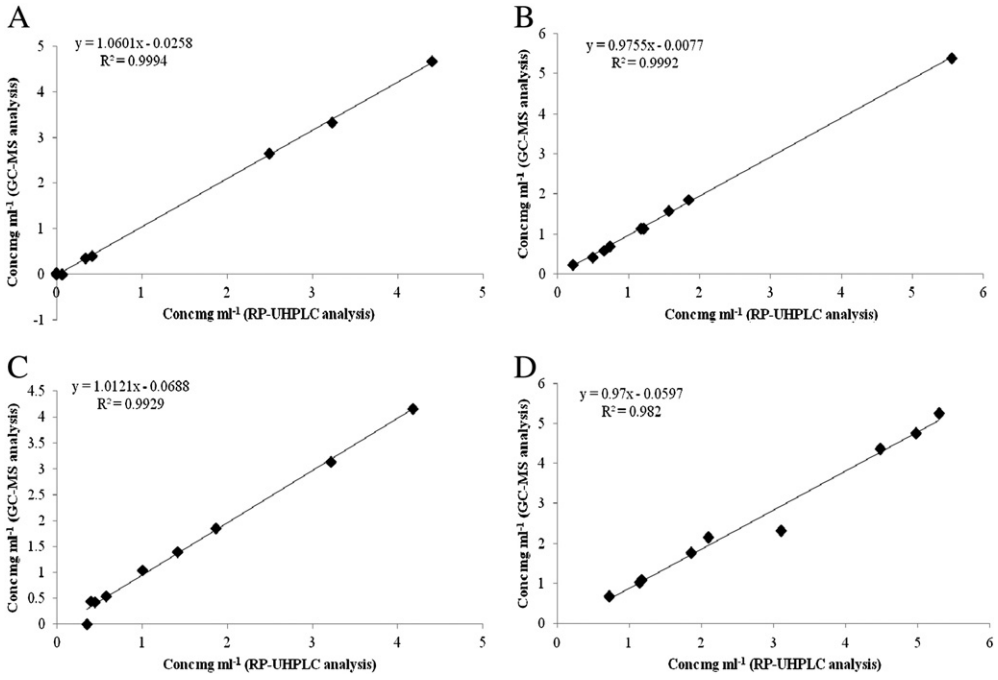

Fig. 3. Correlation between RP-UHPLC PDA and GC–MS results for *Sceletium tortuosum* raw materials and commercial products. A) Mesembrenol; B) mesembranol; C) mesembrenone; D) mesembrine.

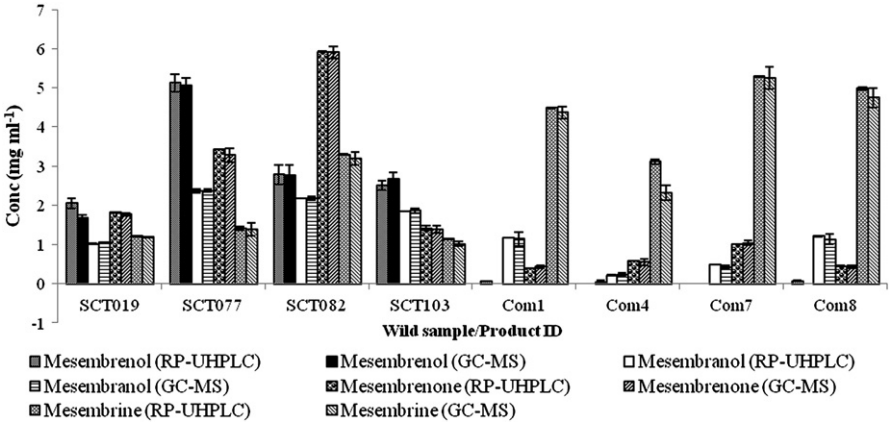

Fig. 4. Quantitative data for alkaloids in raw materials and products as determined by RP-UHPLC PDA and GC–MS analyses.

quantitative determination of four pharmacologically active alkaloids in *S. tortuosum* raw materials and products. It was possible to determine lower levels of the alkaloids by using the developed RP-UHPLC PDA method, rather than the GC–MS method. Quantitative data indicated a linear relationship between the two methods, thus indicating that the two methods are reliable for authentication of *Sceletium* products. These methods are suitable for assay and quality control of *Sceletium* raw materials and products, but could also be valuable tools for the chemotaxonomic evaluation of the species.

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