

Short communication

# A capillary zone electrophoresis method for the assay and quality control of mesembrine in *Sceletium* tablets

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## Abstract

The *Sceletium* plant has been reported to contain psychoactive alkaloids, specifically mesembrine, mesembrenone, mesembrenol and other related alkaloids. *Sceletium* is marketed through health shops and on the internet as dried plant powder and as pharmaceutical dosage forms. The objectives of this research was to develop and validate a capillary zone electrophoresis (CZE) method to identify five alkaloids and quantitatively determine the content of the important alkaloid, mesembrine in *Sceletium* tablets. Since reference standards of the relevant alkaloids are not commercially available for use in quality control of *Sceletium* products, it was necessary to isolate and characterize an appropriate analytical marker for use in the assay and additional markers for fingerprinting by CZE. The separation of the relevant alkaloids was carried out by CZE on a 50 cm effective length, fused silica capillary tubing (50  $\mu\text{m}$  i.d.  $\times$  360  $\mu\text{m}$  o.d.) using 50 mM of sodium dihydrogen orthophosphate dihydrate at pH 1.5 as the background electrolyte and monitored at a UV wavelength of 228 nm. All the marker alkaloids were found to be well resolved and were identified in the plant material and in commercially available *Sceletium* tablets based on the relative migration times (MTs) with respect to quinine hydrochloride that was used as an internal standard. The method was validated and used to assay the mesembrine content in *Sceletium* tablets. Calibration curves were found to be linear over the entire concentration range of 2.5–80  $\mu\text{g}/\text{ml}$  with correlation coefficients  $>0.995$ . The accuracy was found to be 92.5 and 104.5% (R.S.D.  $<3.5\%$ ) and the R.S.D.'s of the inter-day precision at low, medium and high tablet masses were better than 0.9, 2.2 and 2.7%, respectively.

The recoveries were all within the range of 91.8 and 105.8% (R.S.D.  $<8.5\%$ ) and the limit of quantitation (LOQ) and limit of detection (LOD) values were found to be 2.5 and 1.5  $\mu\text{g}/\text{ml}$ , respectively.

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**Keywords:** *Sceletium* tablets; Capillary zone electrophoresis; Mesembrine; Assay and quality control; Fingerprinting

## 1. Introduction

The use of natural products as complementary and alternate medicines (CAMs) has gained tremendous popularity. Also known as herbal medicines, they have been used in primary health care by the poor in many developing countries and have also gained much acceptance even in countries where orthodox/conventional medicines (allopathic) are the predominant form of drug therapy. However, the use of such medicines varies according to specific regions and cultures around the world, which makes this form of treatment somewhat inconsistent. Safety and efficacy are major concerns due to poor documen-

tation and a dearth of scientific research on this subject [1]. In most instances, selective analytical methods and monographs do not yet exist. A large proportion of such herbal medicines consist of complex mixtures of many constituents where more often than not, the active component may not be known. Issues of the quality of such medicines and associated quality control therefore present difficult and demanding challenges to regulatory authorities. Hence when several constituents have to be considered simultaneously, standardization becomes even more complicated because of the variation in the ratios of relevant constituents in different batches [2].

The genus *Sceletium* belongs to the family, Aizoaceae, which occurs in the Western, Eastern and Northern Cape province of South Africa, more prominently occurring in the Little, Great and Upper Karoo regions. It is also reported to be found in the Namaqualand Rocky Hills, Knersvlakte, Ceres Karoo and

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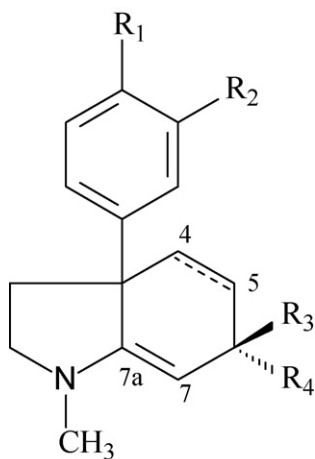


Fig. 1. 3a-Aryl-*cis*-octahydroindole skeleton.

some moister parts of Western Cape [3]. The historical and traditional preparation of this plant has been reported as *Kougoed* or *Channa*, which refers to a preparation method used by the native Bushmen of Namaqualand. *Sceletium* is used for its psychoactive preparations and historically by the Khoisan tribe of southern Africa. Reports of trade and commercial interests of this plant, date back as early as the 17th century [4]. The traditional preparation is made by a fermentation process, which is purported to enhance the stimulant effect of the plant [4].

Of the alkaloids which have been isolated from *Sceletium* species, mesembrine alkaloids are reported as the major subgroup which has the 3a-aryl-*cis*-octahydroindole skeleton which includes the  $\Delta^4$ - and the  $\Delta^7$  mesembrine-type series based on the double bond at either position 4–5 or 7–7a, respectively [5] (Fig. 1).

The major alkaloid is (–)-mesembrine (Fig. 2a), reported to be present in up to 1% in *S. namaquense* and occurs as a partial racemate in *S. strictum* and *S. tortuosum* in smaller amounts [6]. The other alkaloids, namely mesembrenone,  $\Delta^7$  mesembrenone, mesembranol and epimesembranol (Fig. 2b–e) are also present in the plant and were found to be in lower quantities during this study.

Capillary electrophoresis (CE) is a technique where charged components are separated in small capillaries based on their electrophoretic mobilities in an electrolyte under the influence of an applied electric field. This technique permits an efficient and rapid separation of compounds resulting in a relatively fast analysis [7]. CE is a relatively new analytical technique which has

gained popularity over the last two decades [8], mainly due to its wide application, high efficiency, short analysis times and importantly, low cost. This technique is well suited for the analysis of natural products which generally contain multiple and complex chemical constituents that usually require lengthy analysis times and complex separation methods [9].

Alkaloids, being strong bases [10], are good candidates for CE analysis as they are readily protonated and provide positive charge in solutions of low pH [9]. Using this technique, many alkaloidal compounds have been studied and reported [9,11].

General approaches for alkaloidal analysis using CE coupled with MS have been reported for indole alkaloids [11] as well as a report on the influence of alkaloidal structure on electrophoretic mobility [10]. CZE methods, to our knowledge, have not yet been reported in the scientific literature for *Sceletium* alkaloids.

## 2. Experimental

### 2.1. Reagents and chemicals

HPLC grade methanol (Romil Ltd., Cambridge, Great Britain) was used and all the other reagents and chemicals, sodium dihydrogen orthophosphate dihydrate (Saarchem (Pty) Ltd., Muddersdrift, South Africa), sodium hydroxide (NaOH) (Associated Chemical Enterprises (Pty) Ltd., Southdale, South Africa), orthophosphoric acid ( $H_3PO_4$ ) (Merck Chemicals (Pty) Ltd., Wadeville, South Africa) were of laboratory grade. Water was purified in a Milli-Q<sup>®</sup> system, Millipore (Bedford, USA). *Sceletium* reference compounds mesembrine, mesembrenone and  $\Delta^7$  mesembrenone were isolated from plant material and the compounds mesembranol and epimesembranol were produced by hydrogenation of mesembrine and purified. All compounds were subjected to analysis by  $^1H$ ,  $^{13}C$  and 2D NMR [12]. The internal standard (IS) quinine hydrochloride (Q HCl) was procured from Sigma Chemical Company (St. Louis, MO, USA) and *Sceletium* tablets were purchased from a local pharmacy in Grahamstown, South Africa.

### 2.2. Equipment and capillary electrophoretic conditions

The CE instrumentation comprised a PrinCE (4tray) CE System Model 0500-002/OR and diode array detector DAD-160, Model 0005-133. The CE system, detector operation and data processing were achieved by software DAX3D Data Acquisition

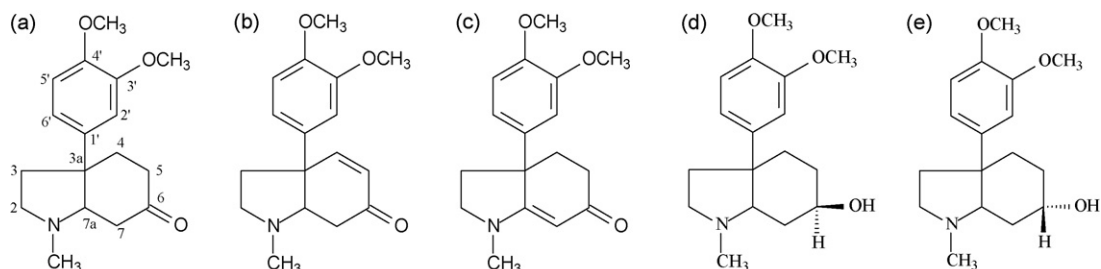


Fig. 2. Mesembrine (a), mesembrenone (b),  $\Delta^7$  mesembrenone (c), mesembranol (d), and epimesembranol (e).

and Analysis Version 8.0 (Prince Technologies B.V., Emmen, The Netherlands). A Linear UV/vis Model 200 ultraviolet detector (Linear Instruments Corp., Reno, NV, USA) was used to monitor the compounds at a UV wavelength of 228 nm and the data output from this detector was interfaced through a SATIN<sup>®</sup> box, to a Waters<sup>®</sup> Empower Chromatographic Manager (Waters Chromatography Division, Milford, MA, USA). NMR analysis was performed on a Bruker Advance DRX 400 MHz NMR Spectrometer (Rheinstetten, Germany). The separation of alkaloids was carried out on a 50 cm effective length, fused silica capillary tubing (50  $\mu\text{m}$  i.d.  $\times$  360  $\mu\text{m}$  o.d.) obtained from Polymicro Technologies, L.L.C. Phoenix, Arizona, USA. A Cole-Parmer ultrasonic bath, Model 8845-30 (Cole-Parmer Instrument Company, Chicago, IL, USA) was used during sample extraction and a Crison GLP21 pH Meter (Crison, Barcelona, Spain) was used to measure and adjust the pH of the relevant solutions.

Capillaries of 65 cm were cut and an effective length to the window of 50 cm was made with a gas flame. New capillaries were conditioned with 1 M NaOH solution for 30 min, 0.1 M NaOH solution for 30 min and then water for 40 min using a pressure of >2500 mbar whereas washing of the capillaries between consecutive injections to ensure optimal charge density on the capillary wall during analytical work was done with water for 4 min, 1 M NaOH for 2 min, 0.1 M NaOH for 2 min and finally with water for 5 min. All capillaries were conditioned with background electrolyte for 5 min prior to sample injection. The electrolyte solutions at the anode and cathode were replaced after each injection.

During the course of this study, the initial method development was carried out using a diode array detector (DAD-160 detector and DAX3D software) to obtain the UV spectra of each of the relevant chromatographic peaks and subsequently method validation was carried out daily on three separate days using a Linear UV/vis detector which provided increased sensitivity.

### 2.3. Method development

A simple orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) background electrolyte was used initially and systematic studies were conducted by using 25, 50, 100 and 200 mM solution of  $\text{H}_3\text{PO}_4$  with the applied voltage varying between 15 and 20 kV. A mixture of five alkaloids were injected to confirm the resolution between mesembrine and epimesembranol which showed poor resolution with MT 7.618 min for epimesembranol + mesembrine. The other alkaloids were well-resolved with MTs 7.480, 7.818, 8.365 min for mesembrenone, mesembranol and  $\Delta^7$ mesembrenone, respectively (Fig. 3).

These studies showed a generated current between 17.5 to 95  $\mu\text{A}$ . By increasing the electrolyte concentration to 200 mM  $\text{H}_3\text{PO}_4$ , standards injected at +20 kV which generated a current of  $\sim 95 \mu\text{A}$  resulted in a very slight improvement of the separation where the MT's were 10.037 and 10.107 for epimesembranol and mesembrine, respectively (Fig. 4).

Although the method using  $\text{H}_3\text{PO}_4$  as the electrolyte solution resulted in partial resolution between the various alkaloids and internal standard, the current that was generated during the analysis was very high. This can lead to undesirable joule heating

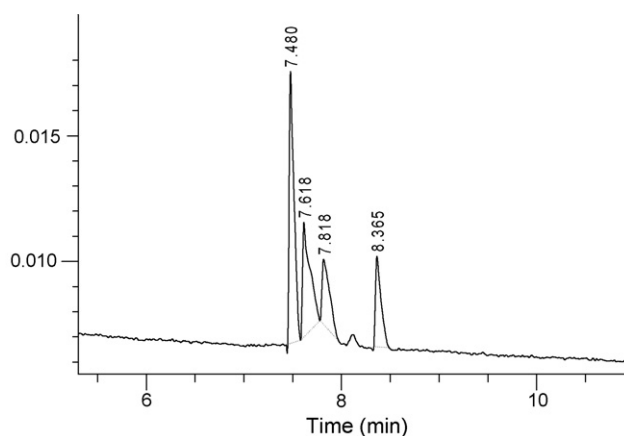


Fig. 3. Electropherogram of standard alkaloids injected at +20 kV with BGE of 100 mM  $\text{H}_3\text{PO}_4$ . Order of elution: mesembrenone 7.480, epimesembranol + mesembrine 7.618, mesembranol 7.818 and  $\Delta^7$ mesembrenone 8.365.

and have deleterious consequences. In view of the need to keep joule heat at a minimum for effective resolution, a solution containing  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (50 mM) as the background electrolyte (BGE) was used to reduce the current. Systematic studies were conducted using a 65 cm effective length capillary at acidic pHs between 4.5 and 1.5 by adjusting the BGE pH using  $\text{H}_3\text{PO}_4$  (Fig. 5a–d).

The optimized electrophoretic conditions were as follows:

- Ambient temperature of  $22 \pm 2^\circ\text{C}$ .
- 50 cm effective length capillary.
- Applied voltage of +16 kV with a voltage ramp of +6 kV/s.
- BGE consisting of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (50 mM) and pH adjusted to 1.5 with  $\text{H}_3\text{PO}_4$ .

Samples were injected electrokinetically at 2 kV for 0.2 min. The alkaloids were well resolved and the migration times were observed at 8.061, 12.004, 12.202, 12.431, 12.660 and 13.556 min corresponding to Q HCl, mesembrenone, epime-

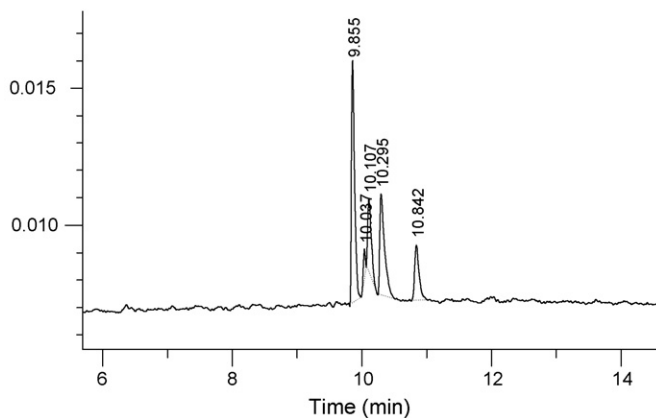


Fig. 4. Electropherogram of standard alkaloids injected at +20 kV with BGE of 200 mM  $\text{H}_3\text{PO}_4$ . Order of elution: mesembrenone 9.855, epimesembranol 10.037, mesembrine 10.107, mesembranol 10.295 and  $\Delta^7$ mesembrenone 10.842.

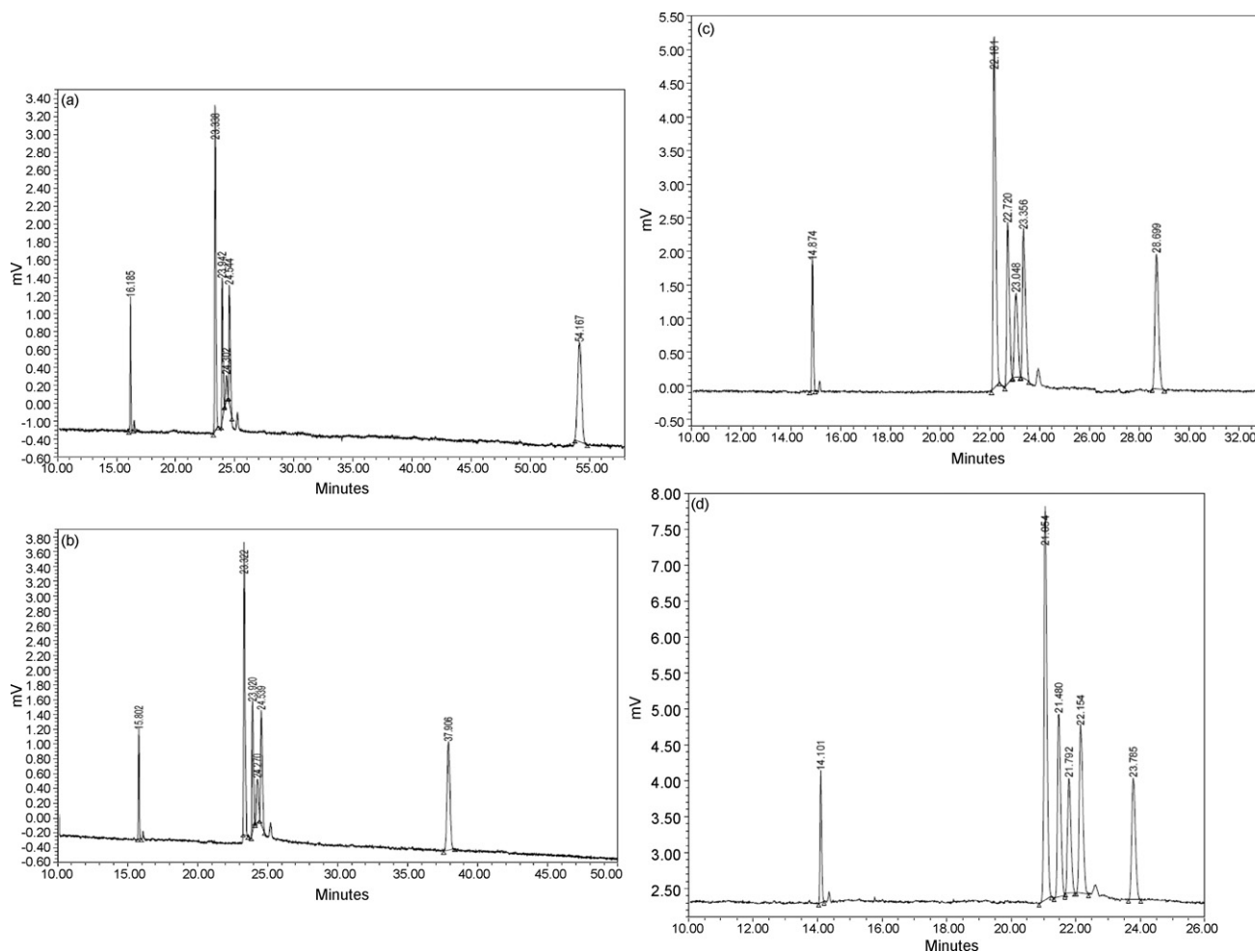


Fig. 5. (a) Electropherogram showing separation of standard alkaloids at pH 4.5. Order of elution: Q HCl 16.185, mesembrenone 23.338, epimesembranol 23.942, mesembrine 24.302, mesembranol 24.544 and  $\Delta^7$ mesembrenone 54.167. (b) Electropherogram showing separation of standard alkaloids at pH 3.5. Order of elution: Q HCl 15.802, mesembrenone 23.322, epimesembranol 23.920, mesembrine 24.270, mesembranol 24.539 and  $\Delta^7$ mesembrenone 37.906. (c) Electropherogram showing separation of standard alkaloids at pH 2.5. Order of elution: Q HCl 14.874, mesembrenone 22.181, epimesembranol 22.720, mesembrine 23.048, mesembranol 23.356 and  $\Delta^7$ mesembrenone 28.699. (d) Electropherogram showing separation of standard alkaloids at pH 2.5. Order of elution: Q HCl 14.101, mesembrenone 21.054, epimesembranol 21.480, mesembrine 21.792, mesembranol 22.154 and  $\Delta^7$ mesembrenone 23.785.

sembranol, mesembrine, mesembranol and  $\Delta^7$ mesembrenone, respectively (Fig. 3). The relative migration times were calculated with respect to MT of Q HCl to identify the respective alkaloids in the samples (Figs. 6 and 7).

#### 2.4. Preparation of standard solutions

Standard methanolic stock solutions (1 mg/ml) of  $\Delta^7$ mesembrenone, mesembranol, mesembrenone, mesem-

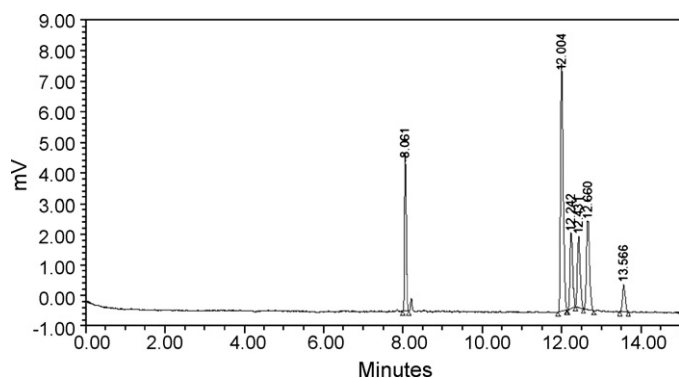


Fig. 6. Typical electropherogram of internal standard with *Sceletium* standards.

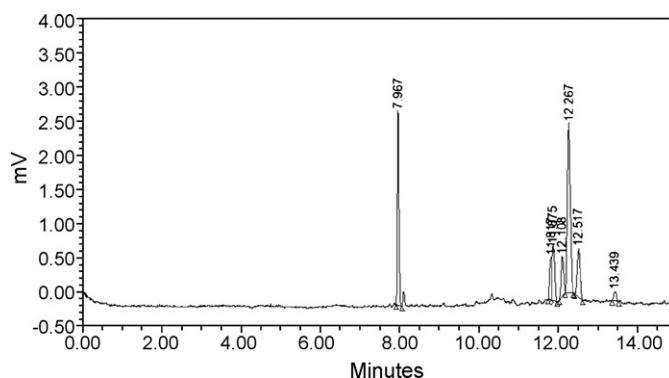


Fig. 7. Typical electropherogram of internal standard and tablet sample.

brine hydrochloride, epimesembranol and Q HCl were prepared.

### 2.5. Linearity

Calibration standards were prepared to obtain six calibrators in the concentration range of 2.5–80  $\mu\text{g/ml}$  of mesembrine hydrochloride. Internal standard (Q HCl in methanol) was added to yield a concentration of 40  $\mu\text{g/ml}$  Q HCl and all standards contained 10% BGE.

### 2.6. Accuracy

Accuracy studies were performed by separately preparing solutions which were appropriately diluted to obtain final concentrations of 20 and 60  $\mu\text{g/ml}$  concentrations of mesembrine hydrochloride for use as QC standards.

### 2.7. Precision

The samples for the precision studies were prepared from commercially purchased *S. tortuosum* tablets (manufactured by Big Tree Health Products, Cape Town, South Africa, Batch no. 9961). Sample solutions containing 25, 50 and 75 mg/ml of tablet powder in triplicate were made up in methanol with the aid of sonication for 20 min. Thereafter, the preparations included

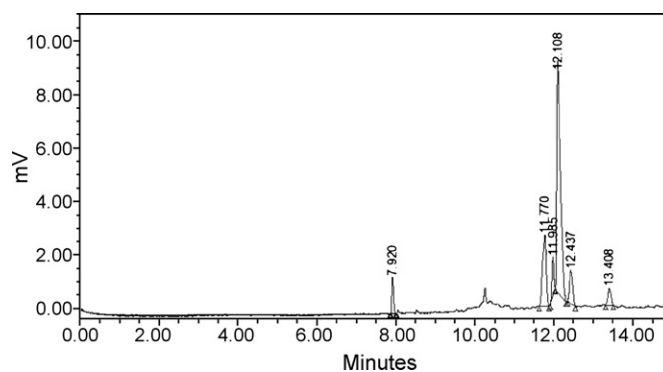


Fig. 8. Typical electropherogram of internal standard and *Sceletium tortuosum* dried plant material.

BGE at a final concentration of 10% in each sample. The samples were filtered through 0.45  $\mu\text{m}$  PVDF membrane filters prior to analysis (Fig. 8).

### 2.8. Recovery studies

Recovery studies were carried out by preparing in triplicate, tablet samples containing 12 mg/ml of tablet powder in methanol. The tablet samples were spiked with low, medium and high concentrations of mesembrine hydrochloride equivalent to 6, 12 and 24  $\mu\text{g/ml}$ , respectively. All samples were prepared in triplicate.

Table 1  
Linearity and coefficients of determination

Name of the compound	Day	$y = mx + c$ (linear model)	Determination coefficient ( $R^2$ )
Mesembrine HCl	1	$y = 0.0353x + 0.0626$	0.9970
	2	$y = 0.0362x + 0.0618$	0.9950
	3	$y = 0.0352x + 0.0621$	0.9964

Slope (S.D.) = 0.000551, intercept (S.D.) = 0.000404.

Table 2  
Accuracy

Mesembrine HCl	Day	Actual weight ( $\mu\text{g/ml}$ )	Calculated weight ( $\mu\text{g/ml}$ )	% Accuracy	Inter-day % R.S.D.
QC 1	1	20.80	20.90	100.50	2.10
	2	20.00	20.90	104.50	
	3	19.20	19.50	101.60	
QC 2	1	62.40	57.70	92.50	3.50
	2	60.00	59.10	98.50	
	3	57.60	53.30	92.50	

Table 3  
Precision studies of *Sceletium* tablets

Compound (mesembrine)	Content) ( $\pm$ S.D.) ( $\mu\text{g}/\text{tablet}$ )			% R.S.D. ( $n = 3$ )
	Day 1	Day 2	Day 3	
Low	162.52 ( $\pm$ 6.51)	162.13 ( $\pm$ 5.65)	164.91 ( $\pm$ 5.60)	0.90
Medium	169.80 ( $\pm$ 0.13)	167.47 ( $\pm$ 1.22)	162.15 ( $\pm$ 3.21)	2.20
High	167.84 ( $\pm$ 10.86)	164.00 ( $\pm$ 12.20)	159.00 ( $\pm$ 6.50)	2.70
Average content	164.30			

Average weight of tablet = 497.3 mg, S.D. = standard deviation. Low = 25 mg/ml ( $n = 3$ ), medium = 50 mg/ml ( $n = 3$ ), high = 75 mg/ml ( $n = 3$ ), total samples  $n = 9$  each day.

Table 4  
Recovery studies of mesembrine from tablet dosage form

Sample	Day 1				Day 2				Day 3				Inter-day recovery % R.S.D. (n=3)
	Mesembrine ((µg/ml added) / (12 mg dosage) ml)	Observed content (µg / (12 mg dosage) ml)	% Recovery	% R.S.D. (n=3)	Mesembrine ((µg/ml added) / (12 mg dosage) ml)	Observed content (µg / (12 mg dosage) ml)	% Recovery	% R.S.D. (n=3)	Mesembrine ((µg/ml added) / (12 mg dosage) ml)	Observed content (µg / (12 mg dosage) ml)	% Recovery	% R.S.D. (n=3)	
Content <sup>a</sup>	4.02				3.97				4.13				
Low (+5.55) <sup>b</sup> 9.57 <sup>c</sup>		9.25 (±0.51)	96.66	4.90	(+5.44) 9.41	9.12 (±0.37)	96.92	4.10	(+5.12) 9.25	9.12 (±0.40)	98.60	4.40	1.10
Medium (+11.09) 15.11		14.46 (±1.04)	95.70	7.20	(+10.88) 14.85	15.43 (±1.30)	103.91	8.42	(+10.24) 14.37	15.20 (±1.01)	105.80	6.64	5.27
High (+22.19) 26.21		25.02 (±1.36)	95.46	5.44	(+21.76) 25.73	25.30 (±0.90)	98.33	3.60	(+20.48) 24.61	22.60 (±0.81)	91.83	3.60	3.42

<sup>a</sup> Actual content.

<sup>b</sup> Spiked amount.

<sup>c</sup> Represents the total content, i.e. spiked plus original content (follows through the table) (±S.D.), average weight = 497.30 mg/tablet.

### 2.9. Limit of quantitation and limit of detection

A solution of standard stock mixture containing the analyte was diluted appropriately to obtain concentrations for the estimation of LOD and LOQ according to a signal to noise (S/N) ratio of 3:1 and 10:1, respectively.

## 3. Results and discussion

### 3.1. Linearity

Calibration curves were constructed by plotting the peak area ratios of mesembrine/Q HCl versus the concentration corresponding to that analyte on each of 3 days. The curves obtained were found to be linear with determination coefficients greater than 0.99 as shown in Table 1.

### 3.2. Accuracy

The studies were performed using QC samples that were prepared separately on each of the 3 days of the analyses. The inter-day R.S.D. values obtained were less than 4% for the mesembrine QC standards. The accuracy of the method was found to be between 92.50 and 104.50% (R.S.D. < 3.5%) (Table 2).

### 3.3. Precision

The average content of mesembrine per tablet was found to be 164.30 µg and yielded R.S.D.'s of inter-day precision at low, medium and high tablet masses better than 0.9, 2.2 and 2.7%, respectively (Table 3).

### 3.4. Recovery

The values for recovery of mesembrine were between 91.83 and 105.80% over the range of spiked concentrations used with R.S.D.s of less than 8.5% (Table 4).

### 3.5. Limits of detection and quantitation

The LOD (signal/noise > 3) and LOQ (signal/noise > 10) were determined by analyzing serial dilutions of known concentrations of mesembrine HCl solutions. The LOD and LOQ were found to be 1.5 and 2.5 µg/ml, respectively.

## 4. Conclusions

CZE methods for the analysis of *Scelletium* have been conspicuously absent from the scientific literature. In view of the advantages and potential offered by CE, a CZE method was developed and validated for application to fingerprint the presence of alkaloids as well as for use as an assay method for the quantitative analysis of mesembrine, as a marker compound, in *Scelletium* products.

Due to the fact that the CE system involves the use of aqueous-based electrolytes and relatively cheaper uncoated fused silica capillaries for high-resolution separation compared to the more expensive HPLC columns, it is considered as a far more economical procedure which is also easier to transfer between laboratories [13].

Since the identified markers have closely related structures, of which two alkaloids, mesembranol and epimesembranol are epimers, the higher resolution capability of CZE is an attractive incentive to use this method to efficiently separate the multi-component alkaloidal mix in *Sceletium* as well as in accomplishing this in relatively short analysis times. The sample preparations were relatively simple and involved a one step methanol extraction which provided reproducible results. Furthermore, the PDA detector was extremely valuable for peak identification and homogeneity testing during the initial method development.

In summary, a CZE method was developed to identify five alkaloids and quantitatively determine the content of the important alkaloid, mesembrine in *Sceletium* tablets. The method describes a rapid procedure for fingerprinting five alkaloids in *Sceletium* products and has the requisite accuracy and precision for the quantitative analysis of mesembrine in *Sceletium* tablets. These findings indicate that CZE is a valuable alternative and viable option for the fingerprinting and assay of phyto-pharmaceuticals, and as such, can be used as an important QC tool in the quest to assure the quality of CAMs and associated herbal products.

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