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Investigation of the effect of space environment on the contents of atropine and scopolamine in *Datura metel* by capillary zone electrophoresis

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Abstract

The seeds of *Datura metel* were carried aboard a retrievable satellite and exposed to space environment. The effects of space environment (weightlessness and ionizing radiation) on the contents of atropine and scopolamine in *D. metel* were investigated by using an effective capillary zone electrophoresis (CZE) method, which employed 50 mmol/l phosphate buffer (pH 8) containing 10% (v/v) tetrahydrofuran as the running buffer. The results showed that the contents of atropine and scopolamine varied to some extent, and the earth-control group has the lowest content of atropine. However, the variation of atropine and scopolamine contents in three groups was not obvious based on *t*-test. At the same time, the optimization of the separation was discussed in detail and the two compounds were completely separated within 10 min with satisfactory repeatability and calibration linearity.

Keywords: Space environmental effect; Atropine and scopolamine; *Datura metel*; Capillary zone electrophoresis

1. Introduction

Several studies [1–4] reported that space environment has very significant effects on the growth, physical and chemical properties of medicinal plants, when the seeds of plants were carried aboard a retrievable satellite, and planted after returning to earth. Space environmental factors mainly include vacuum, weightlessness and ionizing radiation. The previous investigations in this field focused on the effects of space environment on the growth, and the bioactivity of protein and peroxidase in medicinal plants [1–4], however, few report changes of the active constituents in medicinal plants due to space environmental influence.

Datura metel is a traditional Chinese herbal medicine. Pharmacological investigations [5] have shown that tropane alkaloids, as main components accumulated in *D. metel*, have bioactivities, such as antispasmodic activity, antitussive activity and analgesic activity. For the assay of *Datura* tropane alkaloids, a number of methods have been pro-

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posed, including gas-liquid chromatography [6], high-performance liquid chromatography (HPLC) [7,8] and thin-layer chromatographic (TLC) photodensitometry [8]. However, these methods are time consuming and require relatively large amounts of organic reagent. HPLC method requires ion-pair agents added into mobile phase which tends to denature chromatographic column. The development of capillary electrophoresis (CE) has been reviewed many times [9–12], and clearly it continues to be a very active research area in separation science since this technique often provides higher resolving power, shorter analysis time and lower operation cost than liquid chromatography.

In this work, an effective capillary zone electrophoresis (CZE) methods for the determination of two tropane alkaloids, atropine and scopolamine in *D. metel* is described. After the optimization of such parameters as pH, concentration of running buffer, and organic solvent addition, the contents of the two active constituents in the crude drug of *D. metel* were successfully determined within 10 min. By this method, the effects of space environment (weightlessness and ionizing radiation) on the contents of atropine and scopolamine in *D. metel* were investigated.

2. Experimental

2.1. Equipment

All separations were performed on a BioFocus 3000 CE system (Bio-Rad, USA). A 54.6 cm × 50 μm I.D. An uncoated fused silica capillary (Yongnian Optical Fiber Factory, Hebei, China) was utilized with an effective length of 50 cm, and the temperature was maintained at 25 °C. The other conditions are as follows: applied voltage 15 kV, and UV detection at 210 nm, and samples injection at 50 mbar for 10 s.

The electrolyte solution was 50 mmol/l phosphate buffer (pH 8), containing 10% (v/v) tetrahydrofuran (THF), which was filtered through a 0.45 μm membrane filter and degassed by ultrasonication for approximately 10 min before use. The capillary was conditioned daily by washing first with 0.5 mol/l sodium hydroxide (10 min), then with water (10 min) and finally with the running buffer (15 min). Between consecutive analysis, the capillary was flushed with 0.5 mol/l sodium hydroxide (1 min), then with water (2 min) and finally with the running buffer (3 min) in order to improve the migration time and peak-shape repeatabilities.

2.2. Chemicals

The standard atropine and scopolamine were kindly provided by the Institute of Medicinal Plant Development (Beijing, P.R. China). All chemicals were of analytical-reagent grade: monosodium orthophosphate, hydroxide sodium, THF and methanol from Beijing Chemical Factory (Beijing, P.R. China); pure water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used for all buffer solution.

The seeds of *D. metel* were provided by the Institute of Medicinal Plant Development (Beijing, P.R. China), and were carried aboard a retrievable satellite. After returning to earth, the seeds were divided into two groups: ionizing radiation group with weightlessness effect, and weightlessness group which ionizing radiation could be avoided by coating a layer of anti- ionizing radiation material on the seeds. These two groups of seeds, along with the earth-control group without exposure to space environment, were planted under the same growth conditions. Because there was an oxygen-supply device in the satellite, the effect of vacuum could be excluded.

2.3. Sample preparation

Pulverized dried *D. metel* crude drug of (1.0 g) was extracted with methanol (7 ml) by ultrasonication at room temperature for 30 min, then centrifuged at 1500 rpm for 10 min. The extraction was repeated three times. The extracts were concentrated to 5 ml, and then passed through a 0.45 μm membrane filter.

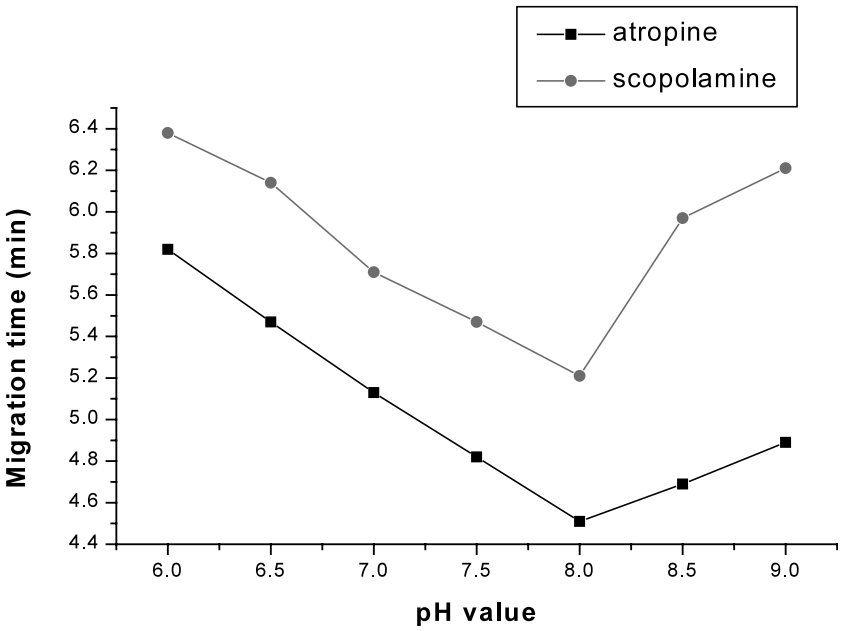

Fig. 1. Plot of migration time vs. running buffer pH for atropine and scopolamine. For separation conditions see Section 2.

2.4. Solutions for construction of calibration curve

Six calibration solutions containing atropine and scopolamine were prepared in methanol with the concentration ranging from 19.4 to 306.0 μg/ml in order to establish a quantitative calibration of these two analytes by the external standard method.

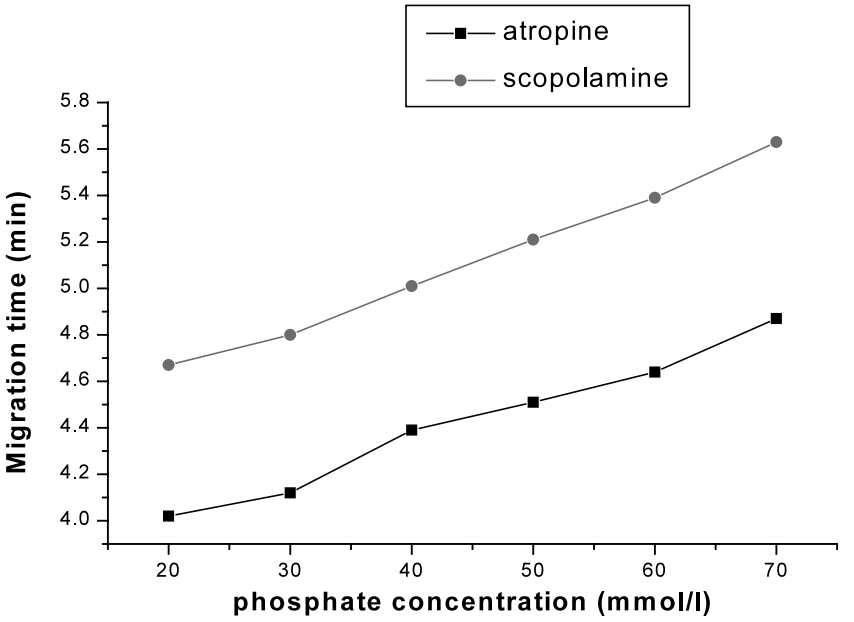

Fig. 2. Plot of migration time vs. phosphate concentration for atropine and scopolamine. For separation conditions see Section 2.

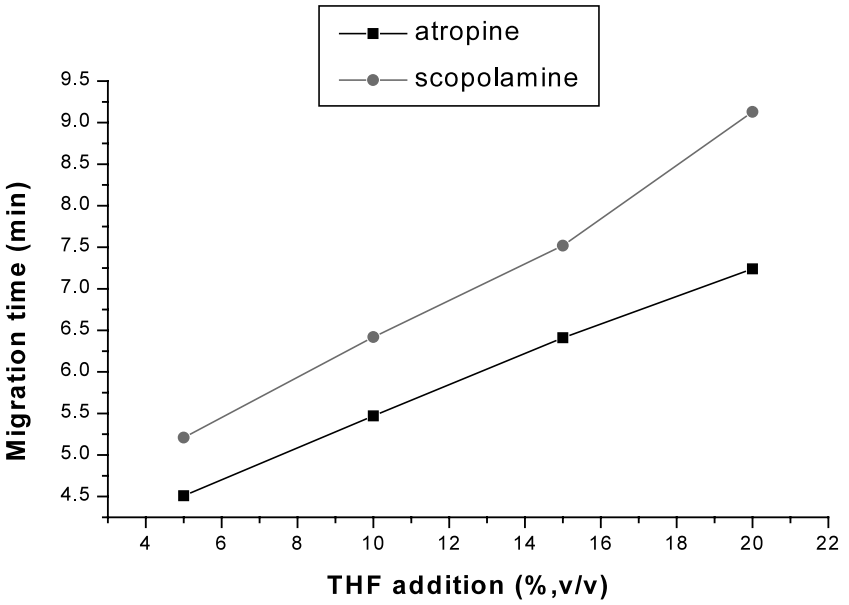

Fig. 3. Plot of migration time vs. THF percentage in the buffer for atropine and scopolamine. For separation conditions see Section 2.

2.5. Solution for recovery testing

Known amounts of atropine and scopolamine were added to the sample of the crude drug of *D. metel* (earth-control group). The mixtures were extracted and analyzed by the same procedures as described above.

3. Results and discussion

3.1. Optimization of analytical conditions

To verify the effect of buffer pH on migration behavior, experiments were performed using 50 mmol/l phosphate buffers at different pH values under 15 kV applied voltage and 25 °C temperature. The experiment showed that the migration times of atropine and scopolamine decrease with the increase of pH from 6 to 8, because of the increase in electroosmotic flow (EOF) at elevated buffer pH, as shown in Fig. 1. At pH 8–9, the migration times of atropine and scopolamine increased with the increase of pH, due to lower protonation of the nitrogen atom of atropine and scopolamine at higher pH resulting in lower mobilities of analytes although EOF increases at higher pH. Accounting for resolution, peak shape, and analytical time, pH 8 was selected for the further optimization.

The effect of phosphate concentration on migration time in the buffer at pH 8 under 15 kV applied voltage and 25 °C temperature indicated that, when the phosphate concentration was varied from 20 to 60 mmol/l, the migration time of atropine and scopolamine increased because EOF is decreased with the increase of phosphate concentration, as indicated in Fig. 2. Finally, with

Table 1
The repeatabilities of peak areas and migration times for atropine and scopolamine ($n = 5$)

Compound	RSD (%) for peak area	RSD (%) for migration time	Detection limit (μg/ml)
Atropine	0.90	1.57	3.2
Scopolamine	1.47	1.82	3.5

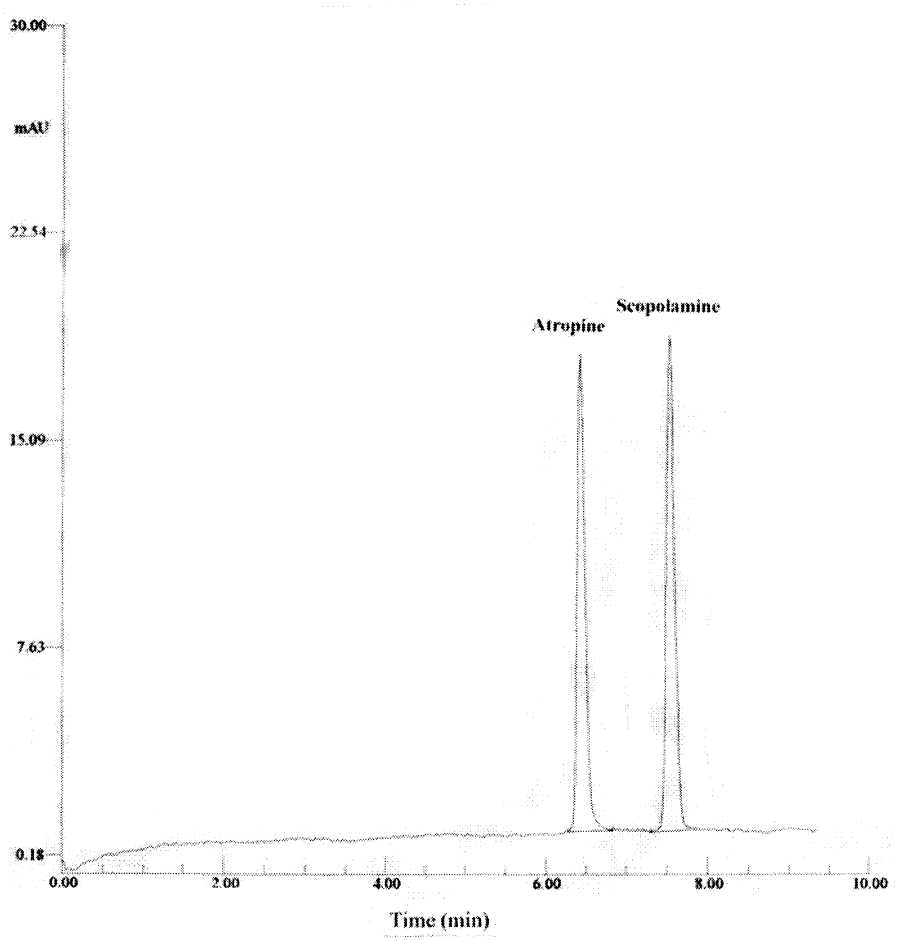

Fig. 4. Electropherogram of mixed standards. For separation conditions see Section 2.

regard to overall resolution and analytical time, 50 mmol/l phosphate buffer was selected for further optimization although the peak shape was not satisfactory at this concentration.

In order to improve the peak shape, 5–20% (v/v) of THF was added into 50 mmol/l phosphate buffer (pH 8), which was used to separate the tropane alkaloids under 15 kV applied voltage and 25 °C temperature. The results showed that, with the addition of THF, the migration times of atropine and scopolamine increase and the peak shape became more symmetric (see Fig. 3). However, with the addition of 15 and 20% THF, the migration times were too long. As peak shape and analytical time were concerned, the 10% (v/v) THF was selected.

Therefore, the 50 mM phosphate buffer (pH 8.0) containing 10% (v/v) THF under 15 kV applied voltage and 25 °C column temperature was

Table 2
The recovery of atropine ($n = 4$) in the earth-control group

No.	Added (mg)[a]	Found (mg)[b]	Recovery (%)
1	0.3672	0.3608	98.3
2	0.3672	0.3705	100.9
3	0.3672	0.3793	103.3
4	0.3672	0.3834	104.4
Average recovery (%)	101.7 (RSD = 2.68%)		

[a] Donates the added amount of atropine standard.
[b] Donates the test amount of atropine in the sample solution for recovery test.

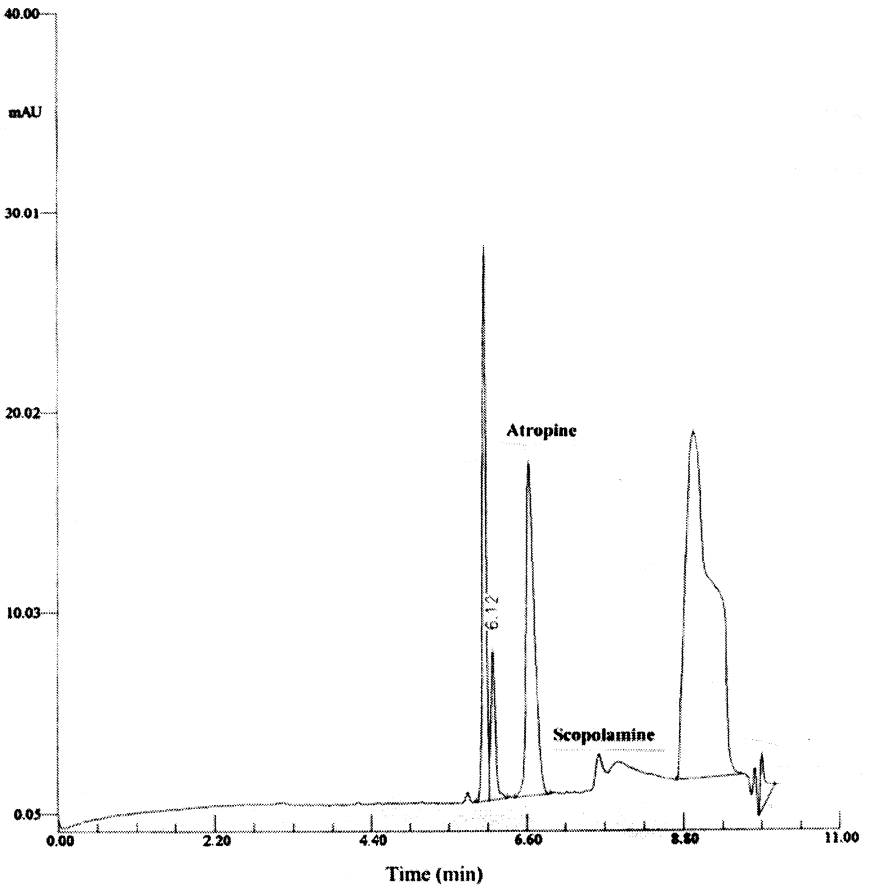

Fig. 5. Electropherogram of the sample from earth control group. For separation conditions see Section 2.

proved to be the optimized condition for the separation. Fig. 4 shows the electropherogram of atropine and scopolamine standards. A typical electrepherogram obtained from the crude drug of *D. metel* is shown in Fig. 5.

3.2. Construction of calibration curves

Calibration curves were constructed in the concentration ranges 24.5–306.0 μg/ml for atropine and 19.4–242.1 μg/ml for scopolamine, respectively. The linear regression equations and correlation coefficients were:

For atropine $Y = 0.1699X - 0.0475$ $(r = 0.9998)$

For scopolamine $Y = 0.2148X - 0.0189$ $(r = 0.9993)$

where X is the peak area of atropine or scopola-

Table 3
The contents of atropine and scopolamine in three groups sample ($n = 3$)

Compound	Earth-control group	Weightlessness group	Ionizing radiation group
Atropine	0.0307% RSD = 3.12%	0.0384% RSD = 2.97%	0.0351% RSD = 2.45%
Scopolamine	Trace	Trace	Trace

mine, and Y is the corresponding concentration (μg/ml).

3.3. System suitability test

The relative standard deviations (RSD) were less than 2.0% for both peak areas and migration times, and the detection limits ($S/N = 3$) for atropine and scopolamine were less than 5 μg/ml. The detailed data are given in Table 1.

The recovery of atropine from *D. metel* was determined by the method of standard addition, and the results are listed in Table 2. The recovery of scopolamine was not obtained due to low concentration in all samples.

3.4. The effects of space environment on the contents of atropine and scopolamine in D. metel

Atropine and scopolamine in *D. metel* can be quantified by the linear regression equation mentioned above, and the results are listed in Table 3. In the experiment, it was observed that scopolamine was interfered by another unknown compound in the sample solution to some extent. The adjustment of THF percentage, applied voltage and temperature had little effect on the separation improvement. However, because the determination of scopolamine was difficult due to its very low content in the sample, the separation was enough for the identification of scopolamine. The investigation shows that the contents of atropine and scopolamine varied to some extent, but only trace scopolamine is detected in all sample groups. The earth-control group has the lowest content of atropine, and the content of atropine in the weightlessness group is higher than that in the ionizing radiation group. However, according to t-test, the variation of atropine concentration in three groups was not obvious. As a preliminary work, the investigation shows the differences in the contents of two active constituents in three groups. The determination of atropine and scopolamine from multiple samples and mathematic statistics are in progress.

4. Conclusions

The contents of atropine and scopolamine in *D. metel* were determined by a CZE method within 10 min under the optimized conditions. The repeatability and recovery for the analytes are satisfactory. By this method, the effects of space environment (weightlessness and ionizing radiation) on the contents of atropine and scopolamine in *D. metel* were investigated. The experiment shows that space environment has some effects on the contents. This method could also be effective for quality control of *D. metel*.

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