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Analysis of Alkaloids in Chinese *Ephedra* Species by Gas Chromatographic Methods

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The six *Ephedra* alkaloids (ephedrine, norephedrine, pseudoephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine) in 12 species of Chinese *Ephedra* collected in 16 districts of China were separated and identified by gas chromatography combined with mass spectrometry following trimethylsilylation of the extract. The alkaloidal concentrations were determined using a gas chromatograph equipped with a nitrogen phosphorus detector. The methods described are sensitive, accurate and simple to perform.

Keywords: Ephedra alkaloids; *Ephedra* species; GC/MS; GC/NPD.

INTRODUCTION

The crude drug Herba Ephedrae (Ma Huang) has long been used in Chinese traditional medicine as a diaphoretic, anti-asthmatic and diuretic as well as for the treatment of bronchitis and acute nephritic oedema. The alkaloids isolated from Herba Ephedrae (the so-called *Ephedra* alkaloids) are used in modern medicine for asthma, influenza and some types of inflammation. The main active alkaloid components consist of three pairs of optically active diastereoisomers—L-ephedrine and D-pseudoephedrine, D-norpseudoephedrine and L-norephedrine, L-methylephedrine and D-methylpseudoephedrine. The quality of the crude drug is evaluated in terms of its alkaloid content, but only a few species of Ephedra Herba have been analysed qualitatively and quantitatively, using appropriate methods, for all six alkaloids.

The techniques that have been used for the quantitative analysis of these alkaloids include gas chromatography (GC) (Yamasaki *et al.*, 1974) and high performance liquid chromatography (HPLC) (Sagara *et al.*, 1983; Moriyasu *et al.*, 1984), but the methods thus described seem to be unsatisfactory with respect to accuracy, resolution or sensitivity since the determination was limited to three, four or, at best, five of the six alkaloids. Recently, Zhang Jiann-sheng established an HPLC method (Zhang Jian-sheng *et al.*, 1988) to determine the contents of the six alkaloids in some Chinese *Ephedra*, but the procedure of sample preparation was very complex and the sensitivity was not high enough for the determination of methylephedrine and methylpseudoephedrine in small amounts. Hence a simple, sensitive, accurate and rapid method for the simultaneous identification and determination of all six alkaloids is still required. The GC methods described in this paper can fulfill these requirements.

The six alkaloids were derivatized by trimethylsilylation of the hydroxy and amino groups using *N*-methyl-*N*-trimethylsilyltrifluroacetamide (MSTFA), and the trimethylsilyl-(TMS)-derivatives of the three pairs of diastereoisomers were successfully separated and identified by a gas chromatographic/mass spectrometric (GC/MS) method. The presence of the six alkaloids in 12 species of Chinese *Ephedra* was confirmed by GC/MS data. The contents were determined by GC using a nitrogen-specific detector.

EXPERIMENTAL

Plant material. The plant samples were collected in various districts in China and were verified by the authors; voucher specimens are stored at the Beijing Medical University. The locations and dates of collection are indicated in Table 2.

Instrumentation. *GC/MS.* A Hewlett-Packard 5890A gas chromatograph interfaced to an HP 5970B series mass selective detector (MSD), HP 7673A autosampler and HP 2934A printer controlled by a HP 59970 ChemStation® was used. A 5% cross-linked phenylmethylsilicone capillary column (HP-5, length 25 m, i.d. 0.2 mm, film thickness 0.33 μm) was used for chromatographic separation. The carrier gas was helium at a flow rate of 0.9 mL/min; injection was performed in splitless mode, and the detector was operated in the scan

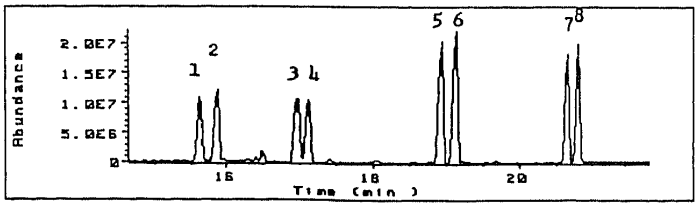

Figure 1. Total ion current chromatogram of TMS derivatives. (1) pseudoephedrine-*O*-TMS, (2) ephedrine-*O*-TMS, (3) methylpseudoephedrine-*O*-TMS, (4) methylephedrine-*O*-TMS, (5) norpseudoephedrine-*O,N*-$(TMS)_2$, (6) norephedrine-*O,N*-$(TMS)_2$, (7) ephedrine-*O,N*-$(TMS)_2$, (8) pseudoephedrine-*O,N*-$(TMS)_2$.

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Table 1. GC/MS data for TMS derivatives of *Ephedra* alkaloids

Derivatives	*RT*(min)	MW	Base peak	Characteristic ions (*m/z*)
Pseudophedrine-*O*-TMS	15.616	237	58	73, 179, 222($M^{+\cdot}-15$)
Ephedrine-*O*-TMS	15.871	237	58	73, 179, 222($M^{+\cdot}-15$)
Methylpseudoephedrine-*O*-TMS	16.983	251	72	73, 163, 236($M^{+\cdot}-15$)
Methylephedrine-*O*-TMS	17.111	251	72	73, 163, 236($M^{+\cdot}-15$)
Norpseudoephedrine-*O,N*-$(TMS)_2$	18.938	295	116	73, 147, 179, 280($M^{+\cdot}-15$)
Norephedrine-*O, N*-$(TMS)_2$	19.137	295	116	73, 147, 179, 280($M^{+\cdot}-15$)
Ephedrine-*O,N*-$(TMS)_2$	20.670	309	130	73, 147, 179, 294($M^{+\cdot}-15$)
Pseudoephedrine-*O,N*-$(TMS)_2$	20.817	309	130	73, 147, 163. 179, 294($M^{+\cdot}-15$)

mode. The electron impact ionization potential was 70 eV, the injector temperature 220 °C and the interface temperature 280 °C. The temperature programme was as follows: initial temperature 100 °C held for 1 min, increased by 3 °C/min to 150 °C and then to 280 °C at a rate of 15 °C/min, and finally held for 2 min.

GC/NPD. An HP 5890A gas chromatographs equipped with a nitrogen–phosphorus detector (NPD), HP 3393A integrator and an HP 7673A autosampler, was employed. An HP-5 fused silica capillary column (length 25 m, i.d. 0.2 mm, film thickness 0.33 μm) was used with detector temperature at 280 °C, injector temperature 220 °C, and oven temperature initially at 90 °C, held for 1 min and increased by 3 °C/min to 124 °C, held for 3 min and then to 280 °C at a rate of 20 °C/min. The carrier gas was helium at a flow rate of 1.4 mL/min, the hydrogen flow was 3.2 mL/min, the air flow was 90 mL/min, the make-up gas was 27 mL/min and the split ratio was 1:10.

Reagents. Ephedrine hydrochloride, pseudoephedrine hydrochloride, norpseudoephedrine hydrochloride, norephedrine hydrochloride and methylephedrine hydrochloride were obtained in either "Pharmaceutical" or "Analar" grades (Serva or Aldrich); methylpseudoephedrine was purified by column chromatography and recrystallization from the crude preparation obtained from Ephedrae Herba, and identified by melting point, HPLC and GC/MS. MSTFA was AR grade (Pierce), diethyl ether was distilled over CaH_2, diphenylamine solution (10 ppm) was prepared by dissolving 5 mg of diphenylamine in 500 mL of redistilled diethyl ether; stock standard solutions of alkaloids contained 1 mg/mL (calculated as base) in methanol. All other reagents were of analytical grade.

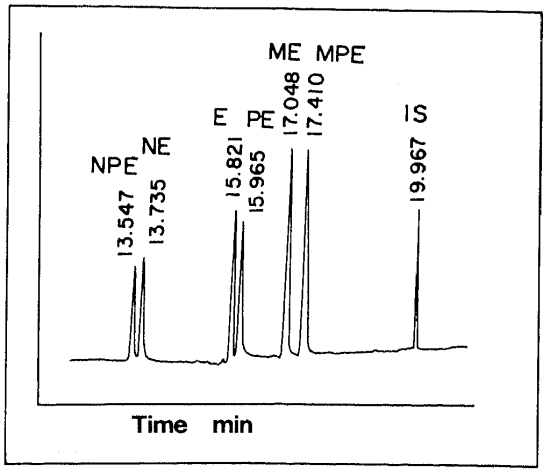

Figure 2. Chromatogram of *Ephedra* alkaloids. NPE: norpseudoephedrine, NE: norephedrine, E: ephedrine, PE: pseudoephedrine, ME: methylephedrine, MPE: methylpseudoephedrine, IS: internal standard.

Identification. An aliquot (25 mg) of the powdered drug was weighed and transferred to a 10 mL centrifuge tube and mixed with 3 mL of 0.5 M H_2SO_4 solution. The mixture was shaken for 15 min and then centrifuged for 7 min at 1600 × g. The supernatant layer (2 mL) was transferred to another tube and 0.7 mL of 5 M KOH solution, 1.2 g NaCl and 2 mL of redistilled diethyl ether were added to the tube. The mixture was shaken for 15 min and then centrifuged at 1600 × g for 7 min. The ether layer was taken into a 2 mL sample vial and evaporated to dryness at room temperature under a slow stream of nitrogen. MSTFA (100 μL) was added to the residue, the mixture was reacted at 75 °C for 15 min and 1 μL of the resulting solution was submitted to GC/MS analysis.

Determination. Crude drug (25 mg) was weighed into a 10 mL centrifuge tube and 2 mL of 0.65 M KOH solution, 1.2 g of NaCl and 2.0 mL of diethyl ether containing 10 ppm diphenylamine were added to the tube. The mixture was shaken for 15 min and then centrifuged for 7 min at 1600 × g. The organic layer was pipetted into a 2 mL sample vial containing 0.1 g of anhydrous Na_2SO_4 and the extract solution (2 μL) was submitted to GC/NPD analysis. The concentrations of the six alkaloids in Herba Ephedrae were calculated according to the regression equations deduced using appropriate standards.

RESULTS AND DISCUSSION

It is difficult to identify the *Ephedra* alkaloids by GC according to their retention times, or to distinguish the three pairs of diasteroisomers by GC/MS using normal methods. However, the TMS derivatives of the alkaloids were well separated and identified by the GC/MS method (Fig. 1 and Table 1). The new GC/NPD method employes a simpler extraction procedure than the HPLC method and shows much higher sensitivity.

Table 2. Contents of *Ephedra* alkaloids[a] in Herba Ephedrae (expressed as %)

Species	Place and time of collection	NPE	NE	E	PE	ME	MPE	Total
E. sinica Stapf	Dataong, Shanxi (9/1983)	0.110	0.040	0.760	0.280	0.069	trace	1.259
	Huai-an, Hebei (8/1983)	0.142	0.102	0.805	0.276	0.053	trace	1.378
E. equisetina Bunge	Zhaosu, Xinjiang (8/1986)	0.155	0.198	1.250	0.580	0.025	ND[b]	2.208
E. intermedia Schrenk ex Mey.	Urumchi, Xinjiang (8/1983)	0.111	0.075	0.550	0.912	0.026	ND	1.674
	Datong, Qinghai (8/1983)	0.113	0.063	0.140	0.798	0.013	0.009	1.136
	Dingxi, Gansu (8/1983)	0.130	0.030	0.134	0.805	0.013	0.008	1.120
E. intermedia var. *tibetica* Stapf	Jiacha, Xizang (7/1984)	0.026	0.040	1.150	0.084	0.170	ND	1.470
E. likiangensis Florin	Ludian, Yunnan (9/1986)	0.170	0.051	0.630	0.606	0.027	trace	1.484
E. monosperma Gmel. ex Mey.	Abazhou, Sichuan (9/1983)	0.330	0.180	1.401	0.860	0.054	0.005	2.830
E. minuta var. *dioeca* C. Y. Cheng	Kangding, Sichuan (7/1986)	0.069	0.075	0.722	0.235	0.041	trace	1.142
	Daofu, Sichuan (8/1984)	0.016	0.047	0.371	0.220	0.054	ND	0.708
E. gerardiana Wall.	Lhasa, Xizang (7/1984)	0.074	0.078	0.765	0.101	0.040	trace	1.058
E. saxatilis Royle ex Florin	Lhasa, Xizang (7/1984)	0.023	0.063	0.590	0.050	0.063	ND	0.798
E. lomatolepis Schrenk	Kashi, Xinjiang (7/1982)	0.320	0.037	0.167	0.830	0.005	trace	1.359
E. lepidosperma C. Y. Cheng	Mt. Helan, Ningxia (6/1984)	0.007	0.005	0.012	0.017	0.001	trace	0.042
E. przewalskii Stapf	Qitai, Xinjiang (8/1983)	0.005	0.003	0.029	0.006	0.003	ND	0.046

[a] NPE: norpseudoephedrine, NE: norephedrine, E: ephedrine, PE: pseudoephedrine, ME: methylephedrine, MPE: methylpseudophedrine.
[b] ND: none detected.

Furthermore, the smallest quantity of alkaloid that may be detected by this technique is less than 2 ng and the method is sufficiently sensitive to enable the successful quantification of methylephedrine and methylpseudoephedrine in plant material, even when present in very small amounts.

The occurrence of all six alkaloids in Herba Ephedrae was confirmed by comparison of their GC/MS data with those of the corresponding standards (Table 1). The TMS derivatives were more thermostable and gave better sensitivity and, chromatographic behaviour and more characteristic ions in the mass spectra than the corresponding bases. The *Ephedra* alkaloids and their TMS derivatives showed α-cleavage of amino compounds to yield the corresponding fragment as the base peak. The mass spectra of the O,N-$(TMS)_2$ derivatives of ephedrine and norephedrine produced base peaks of m/z 130 and 116, respectively.

For quantitative analysis, suitable amounts of the stock standard solutions of the alkaloids were treated according to the preparation procedure described for the crude drug. Appropriate calibration curves were constructed for five of the six alkaloids; the regression equations and their correlation coefficients are as follows:

ephedrine $Y = 1.307X - 0.280$ ($r = 0.9991$, range 9–160 ng)

pseudoephedrine $Y = 1.050X - 0.280$ ($r = 0.9990$, range 9–185 ng)

norephedrine $Y = 0.680X - 0.025$ ($r = 0.9997$, range 5–60 ng)

norpseudoephedrine $Y = 0.540X - 0.081$ ($r = 0.9993$, range 5–60 ng)

methylephedrine $Y = 1.357X - 0.036$ ($r = 0.9992$, range 5–60 ng)

where X is the amount ratio ($n = 3$) and Y is the peak area ratio ($n = 3$). The methylpseudoephedrine content in the crude drug was calculated according to the regression equation of methylephedrine because the purity of the sample of the former was not sufficiently high.

Appropriate amounts of the alkaloids were added to samples of the crude drug (the alkaloid content of which was known), and the mixture extracted using the two procedures described in the identification and determination sections. The results showed that there

was no significant difference in the extraction efficiency, but the extraction method described in the determination section is much simpler for GC/NPD determination. The recoveries were 96.6–100.1% (coefficients of variation 2.3–4.7%; $n=6$).

Samples of 12 species of Herba Ephedrae, collected in 16 districts of China, were analysed by GC/MS and GC/NPD methods; the results are shown in Table 2. The results show that ephedrine and pseudoephedrine are the main components in these Herba Ephedrae, but the concentrations of the six *Ephedra* alkaloids vary greatly according to the plant species. The alkaloid contents of *Ephedra sinica*, *E. equisetina*, *E. monosperma* and *E. intermedia* var. *tibetica* are high and ephedrine is the major component. In *E. intermedia* and *E. tomatolepis* the content of pseudoephedrine is higher than that of ephedrine. The content of methylephedrine is higher in *E. intermedia* var. *tibetica* gathered from Xizang (Tibet) and in *E. sinica* from Northeastern China than in the other species.

The results also show that *E. przewalskii* and *E. tepidosperma* contain so little of the *Ephedra* alkaloids (<0.1%) that they are not considered suitable to be used as the drug (Ma Huang).

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