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Short communication

Analysis of tetramethylpyrazine in *Ephedrae herba* by gas chromatography–mass spectrometry and high-performance liquid chromatography

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Abstract

A simple and reliable HPLC method was developed for the determination of 2,3,5,6-tetramethylpyrazine (TMP) in *Ephedrae herba*. Further identification of TMP was achieved using GC–MS. The mobile phase used was methanol–water–35% acetic acid (35:65:0.5, v/v/v) at a flow-rate of 0.8 ml/min. The detection wavelength was set at 290 nm. The linear range of the peak area calibration curve of TMP was 2.64–264 mg/l (r=0.9987) and the recovery for TMP in *Ephedrae herba* extracts was 101.1–106.9%. The relative standard deviations of retention time and peak area were 0.18 and 1.5% (n=6), respectively. The detection limit of TMP was 0.03 mg/l. The contents of TMP in *Ephedrae herba* could easily be determined within 10 min. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pharmaceutical analysis; *Ephedrae herba*; Tetramethylpyrazine; Alkaloids

1. Introduction

The crude drug, *Ephedrae herba* (*Ma Huang*), is contained in various Chinese herbal preparations and has been utilized for perspiratory, antitussive, antipyretic and anti-inflammatory purposes [1]. As a result of pharmacological investigations of the Ephedra herb, it has been shown that most of the clinical efficacy is exerted by its alkaloidal components. The main ingredients of alkaloids in this crude drug are ephedrine alkaloids, such as ephedrine, pseudoephedrine and homologous compounds, which have always been the focus of research [2–4]. However, 2,3,5,6-tetramethylpyrazine (TMP) has also been found in *Ephedrae herba* and plays a very important role in relieving asthma [5]. Several methods have been reported for the determination of TMP in *Ligusticum wallichii Franch* (*Chuan xiong*) by UV [6], HPLC [7], GC, and GC–MS [8]. But TMP in *Ephedrae herba* has never been carefully quantified. Though some articles analyzed TMP in *Ephedrae herba* treating it as a kind of volatile oil [5,9,10], it was not an accurate and simple method for TMP quantification in *Ephedrae herba*. Furthermore, the known methods required tedious pretreatment before analysis. This paper described an easy and reliable method for the qualitative and quantitative analysis of TMP in *Ephedrae herba* crude drug by GC–MS and HPLC.

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2. Experimental

2.1. Chemicals and reagents

TMP was purchased from the Corporation of Chinese Biologicals. *Ephedra herba* (crude medicinal material) was collected from Chengdu, Si Chuan Province. All the solvents used were of analytical grade.

2.2. Apparatus and conditions

2.2.1. HPLC

The chromatographic system (HP series 1100) consisted of a quaternary pump, a degasser, a diode array detection (DAD) system and a HP ChemStation for data analysis. Separation was achieved on a Nova-Pak C_{18} column (150×3.9 mm I.D.). The mobile phase consisted of methanol–water–35% acetic acid (35:65:0.5, v/v/v) and the flow-rate was 0.8 ml/min. The mobile phase was filtered by a Millipore vacuum filter system equipped with a 0.45 μm filter before use. The detector was set at 290 nm and the column temperature was 30°C. The injection volume of samples was 20 μl.

The purity of the chromatographic peaks was assessed by a suitable algorithm of the detector software. Calculations were performed by the external standard method.

2.2.2. GC–MS

The GC–MS system consisted of a gas chromatograph (HP 6890), a mass selective detector (HP 5973) and a HP ChemStation for data analysis. The running conditions were as follows:

Separation was performed on a HP-5 MS capillary column (30.0 m×250 μm, 0.25 μm) using the following temperature programme:

$$60°C \xrightarrow{25°C/min} 100°C \xrightarrow{0.5°C/min} 110°C \xrightarrow{1.0°C/min} 120°C$$

Inlet heater temperature: 280°C; MS source temperature: 250°C; EM voltage: 1294 mV. The flow-rate of carrier gas (He) was 1.0 ml/min with the split ratio 30:1. Both full-scan acquisition mode (m/z 35–400) and selected ion monitoring (SIM) mode were used for detection. Samples of 1 μl were injected manually.

2.3. Preparation of Ephedrae herba extracts

2.3.1. HPLC

A 2.0 g sample of crude medicinal material of *Ephedrae herba* was extracted with 10 ml acidified water (adjusted to pH 4.0 with 1.0 mol/L HCl) by refluxing on a water bath at 80°C for 1 h, then was placed in an ultrasonic bath for 10 min. Extraction was repeated three times. The extracting solution was combined and totaled 26.8 ml. The solution was filtered through a 0.45 μm filter for HPLC analysis.

2.3.2. GC–MS

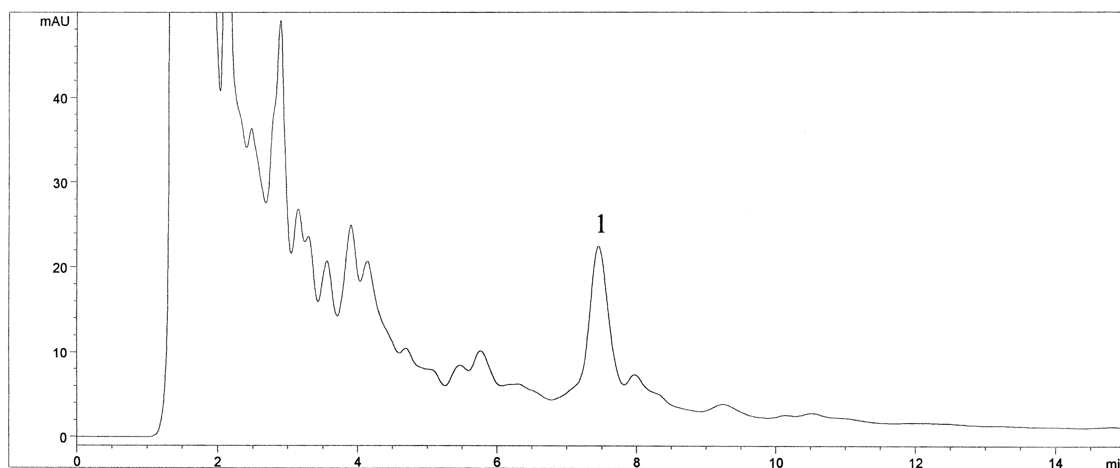
The above acidic solution was adjusted to pH 13 with 25 mol/L NaOH, and then extracted with chloroform. The organic solvent was removed and the residue was dissolved in 10 ml methanol. The methanol solution was filtered through a 0.45 μm filter for GC–MS analysis.

3. Results and discussion

3.1. Identification of TMP in Ephedrae herba

3.1.1. HPLC

The chromatogram corresponding to the *Ephedrae herba* extracts is shown in Fig. 1(a), from which we found that the composition of the extracts was very complex. But most of the main components, such as ephedrine alkaloids, have been eluted before 6 min under the selected HPLC conditions, causing no interference of the determination of TMP. Identification of peak 1 at about 7.9 min as TMP was accomplished by comparing the retention time with that of the TMP standard (Fig. 1(b)) and by comparing the UV spectra (Fig. 2(a)) collected at the apex of the chromatographic peak with the corresponding standard (Fig. 2(b)) recorded under the same conditions. From the comparison of UV spectra, we found that they almost had the same profile with the maximal absorption at about 300 nm. But the peak purity was lower than 95.0%. In fact, it was the resultant of the complex composition of *Ephedrae herba* extracts. Though the chromatographic separation was not sufficient, the choice of 290 nm as detection wavelength made the quantification pos-



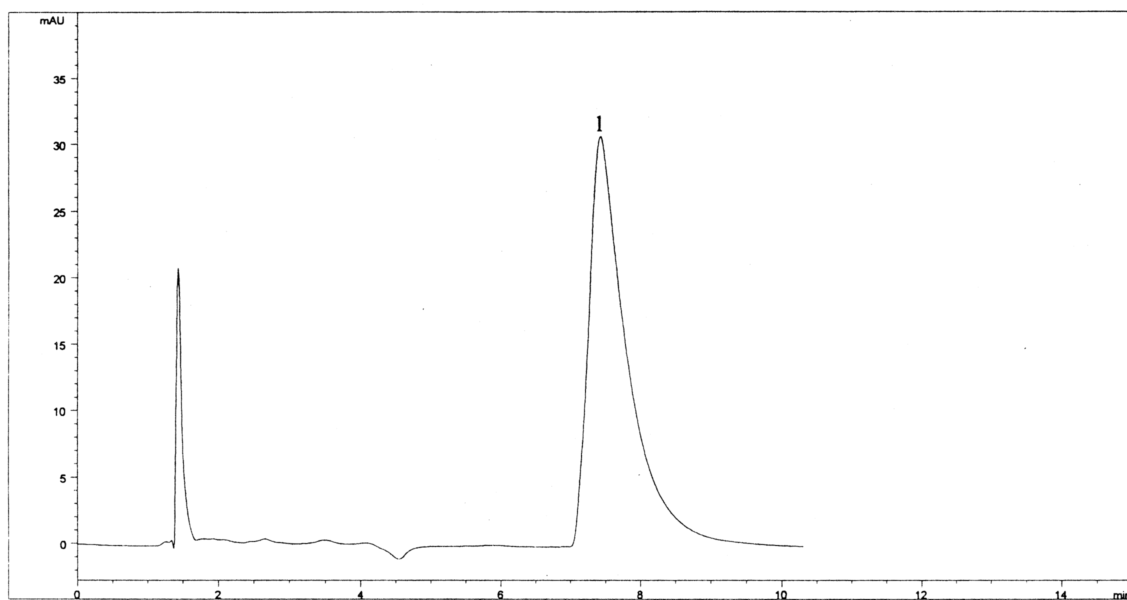


Fig. 1. HPLC Chromatograms of (a) *Ephedrae herba* extracts and (b) TMP standard. 1=TMP.

sible and correct, avoiding the interference of some impurities with absorption at low wavelength.

3.1.2. GC–MS

In order to avoid pollution of the column, we extracted the alkaloidal part out of the direct extracts of *Ephedrae herba* first as described in Section 2.3.2. The total ion current of the extracts is shown in Fig. 3(a). Compared with the retention time of the TMP standard (Fig. 3(b)) and ephedrine (E), pseudoephedrine (PE) standards at the same condition, peaks 1,2 and 3 correspond to TMP, E and PE, respectively.

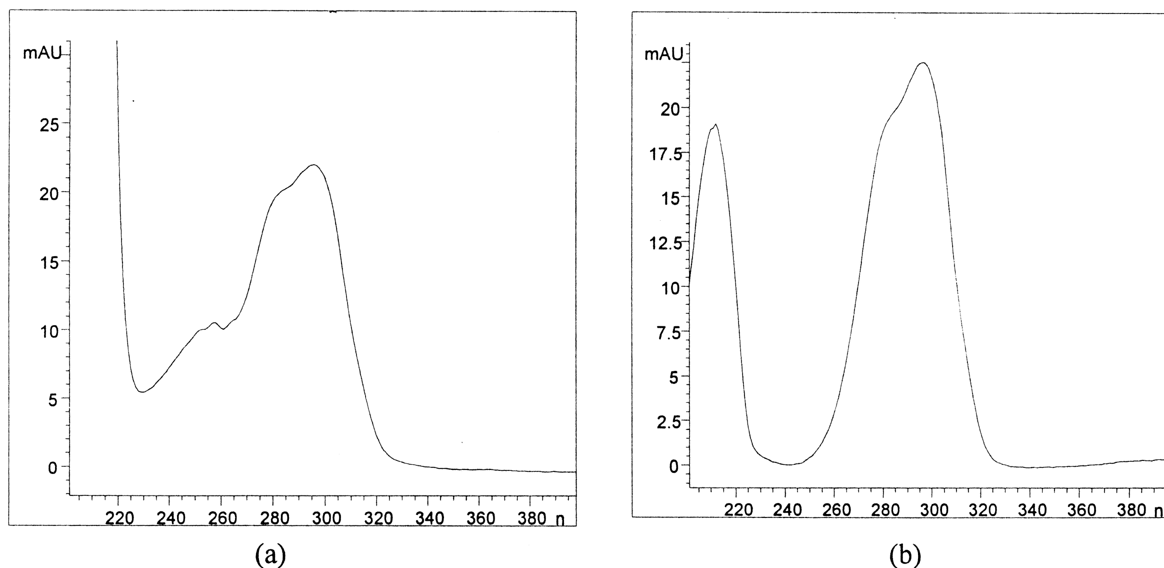


Fig. 2. UV spectra of (a) peak 1 in *Ephedrae herba* extracts and (b) TMP standard.

When using the SIM mode to detect TMP (m/z 136), the sensibility and selectivity were improved significantly, as shown in Fig. 3(c).

For further identification of TMP, the mass spectrum of peak 1 (Fig. 4, upper panel) was compared with that of the TMP standard (Fig. 4, lower panel). It could be seen that they were very similar with the same base peak at m/z 136 and other major peaks at m/z 54 and 42.

So from the information given by HPLC and GC–MS, we could confirm that peak 1 was TMP.

3.2. Linearity and limit of detection

Seven concentrations of TMP methanol solution in the range of 2.64–264 mg/l were prepared for the calibration curve. The peak areas of seven concentrations of TMP were linear related to the concentration of TMP (correlation coefficient, $r=$ 0.99869) and the equation of the regression line was $y=3.17677x+25.24119$. The detection limit for TMP in sample solution was 0.03 mg/l. The detection limit was calculated by the formula: 3 SD/b, where SD is the standard deviation, calculated by injecting six replicates of the lowest concentration solution of the calibration curve, and b is the value of the calibration curve slope. This formula is one of the possible algorithms for calculating the detection limit defined as the analyte amount that gives a signal-to-noise ratio = 3.

3.3. System suitability test

Chromatographic precision of the proposed method, expressed as relative standard deviation (RSD), was calculated by injecting five replicate injections of the central point of the calibration curve. The RSDs of the retention time and the peak areas were 0.18 and 1.5%, respectively (inter-day).

Recovery of the whole analytical procedure was tested with the direct extracts of *Ephedrae herba*. Suitable amounts of TMP standard were added to a sample of *Ephedrae herba* extracts known TMP content and the mixture was analyzed using the proposed procedure. Recovery was expressed, for each component, as the mean percentage ratio between the measured amounts and the actual ones. The standard addition recovery of the TMP was 101.1–106.9%.

3.4. Determination of TMP in Ephedrae herba by HPLC

When the test solutions of *Ephedrae herba* ex-

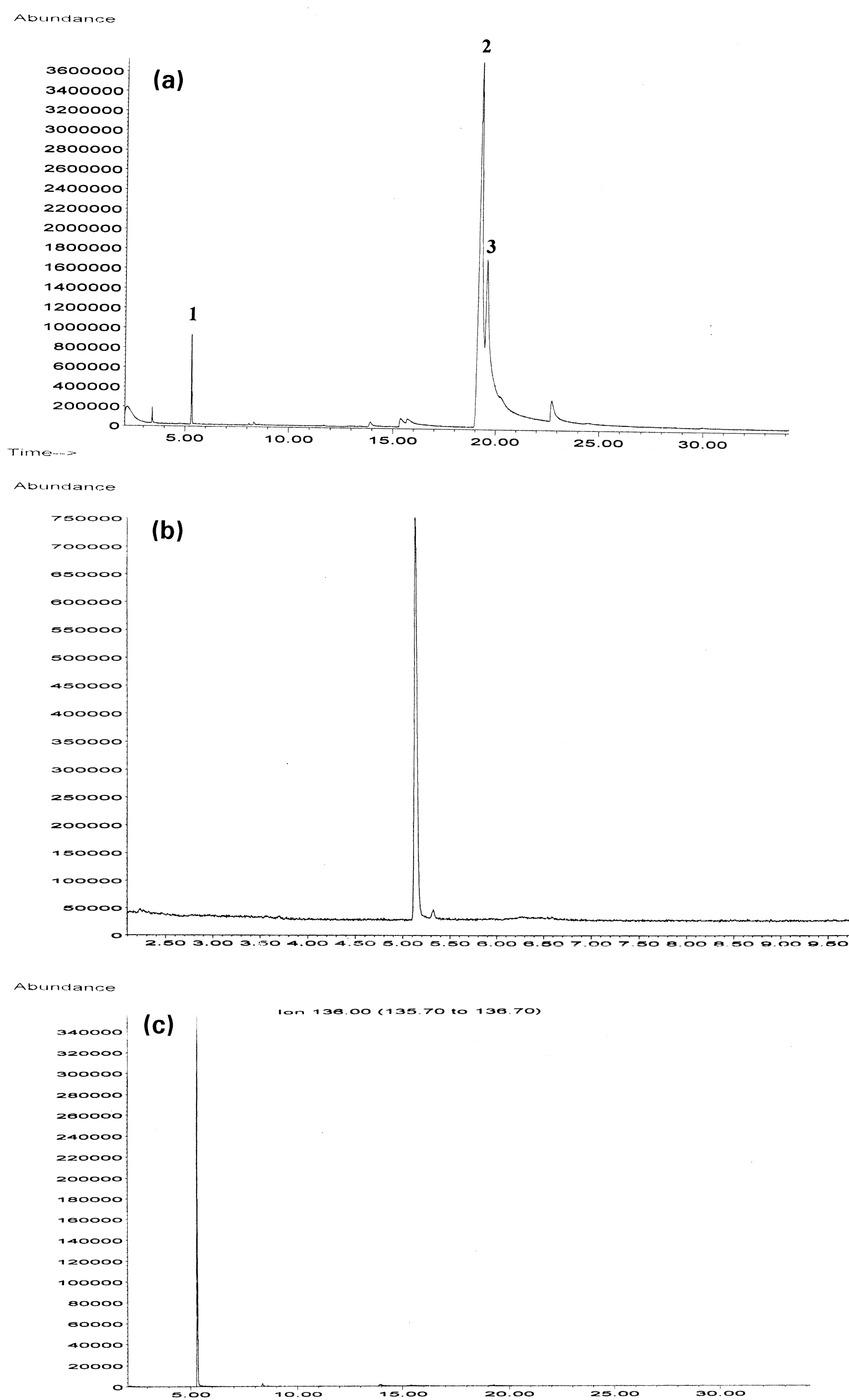


Fig. 3. GC–MS chromatograms of *Ephedra herba* extracts and TMP standard. (a) Total ion current (TIC) of *Ephedra herba* extracts. 1=TMP, 2=E, 3=PE. (b) TIC of TMP standard. (c) *Ephedra herba* Extracts using SIM mode to detect (m/z 136). Time scales in min.

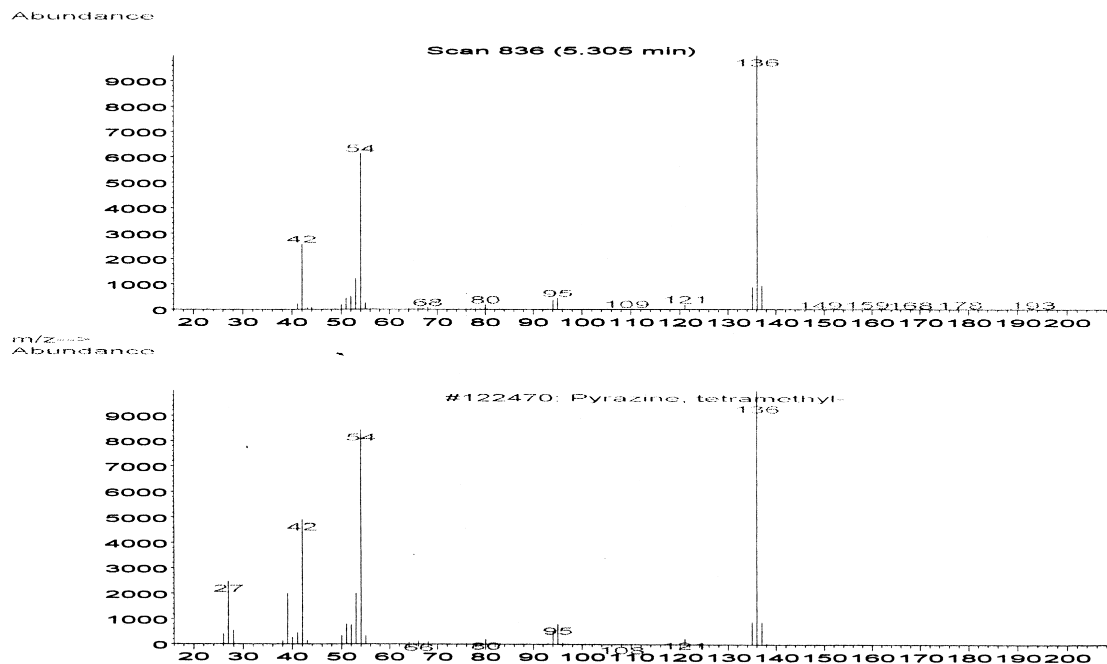


Fig. 4. Mass spectrum of peak 1 in *Ephedra herba* extracts (top) and TMP standard (bottom).

tracts were analyzed by HPLC under the selected conditions, the peak area calibration curves (peak-area, y, vs. concentration, x, mg/l for TMP) were used for quantitative analysis. The calculated content of TMP in *Ephedrae herba* crude drug was 0.2 mg/g.

4. Conclusion

For the first time, TMP in *Ephedrae herba* was analyzed using HPLC, combined with GC–MS identification. The results indicate that the proposed method is suitable for the determination of TMP in *Ephedrae herba*-containing Chinese herbal preparation with rapid and accurate performance. Therefore, it should be useful for quality control in pharmaceutical plants.

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