

*Full Length Research Paper*

# Analysis of the distribution of $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum with gas chromatography–mass spectrometry (GC-MS)

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The  $\beta$ -asarone was a major component of *Acorus tatarinowii* Schott which had significant pharmacological effects on the central nervous system (CNS). We have reported that  $\beta$ -asarone can pass through the blood-brain barrier (BBB) and then go into the brain tissues in the 2002. However, the  $\beta$ -asarone in the brain blood was not excluded, which might lead to a false positive result. In this study, we used the cardio-perfusion method to exclude the  $\beta$ -asarone in the brain blood then examined the brain tissues with gas chromatography–mass spectrometry (GC-MS). Meanwhile we hypothesized that the  $\beta$ -asarone could be widely distributed without target regions in the brain. To test this hypothesis, we analyzed the distribution of  $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum. We found that  $\beta$ -asarone could be detected in rat hippocampus, brainstem, cortex and cerebellum but the  $\beta$ -asarone amount was less than our previous results, which indicated the  $\beta$ -asarone in brain blood had affected the assay of  $\beta$ -asarone in the brain tissues. Meanwhile, we found that distribution of  $\beta$ -asarone among the hippocampus, brainstem, cortex and cerebellum did not have significant differences. We concluded that  $\beta$ -asarone could pass through BBB and be widely distributed without target regions in the brain.

**Key words:** Distribution of  $\beta$ -asarone, *Acorus tatarinowii* Schott, hippocampus, brainstem, cortex, cerebellum, gas chromatography–mass spectrometry (GC-MS).

## INTRODUCTION

The  $\beta$ -asarone is a major component of *Acorus tatarinowii* Schott which has significant pharmacological effects on the CNS (Fang et al., 2008; Cho et al., 2002). It has been reported that  $\beta$ -asarone could attenuate neuronal apoptosis in rat hippocampus and might be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease (Liu et al., 2010; Li et al., 2010). Some other authors found  $\beta$ -asarone could reduce the toxicity of excitatory amino acids in the epileptic rat brain and increase the expression of c-fos (Fang et al., 2008). Additionally,  $\beta$ -asarone could reduce the injuries of blood vessel endothelium and nerve cells

of the cortex (Fu et al., 2008; Chen et al., 2007) and improve the cognitive function of the Beta-amyloid hippocampus injection rats (Geng et al., 2010).

The  $\beta$ -asarone can pass through BBB and then go into the brain as reported by Fang et al. (2002). However, the  $\beta$ -asarone in the brain blood did not exclude, which might lead to a false positive result. In this study, we used the cardio-perfusion method to exclude the  $\beta$ -asarone in the brain blood and examined the brain tissues with GC-MS. Meanwhile, the distribution of  $\beta$ -asarone was measured in rat hippocampus, brainstem, cortex and cerebellum.

## MATERIALS AND METHODS

### Animals

The study and its experimental protocol were approved monitored by the Ethics Committee of Guangzhou University of Chinese

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Medicine. Ten Sprague-Dawley rats (180 to 220 g) were performed according to the guidelines for the ethical treatment of experiment animals. Local institutional approval for research was obtained before initiation of the study.

### The preparation of $\beta$ -asarone

The  $\beta$ -asarone was obtained by four steps. First, the *A. tatarinowii* Schott identified as from the Araceae was purchased from the first affiliated hospital of Guangzhou University of Chinese Medicine. Second, volatile oil was extracted from the *A. tatarinowii* Schott (Chinese Pharmacopoeia Commission, 2010). Third, volatile oil was purified by freezing crystallization and the  $\beta$ -asarone whose purity was up to 99.55% was obtained. The purity of  $\beta$ -asarone was confirmed by the China National Analytical Center with GC-MS, infrared spectrum (IR) and nuclear magnetic resonance (NMR) detection (Figures 1, 2 and 3).

### Experimental design

Ten Sprague-Dawley rats were randomly divided into two groups with two rats in the blank control group and eight the in treatment group. The rats in the treatment group were administered intragastrically in a dose of 200 mg/kg body weight/day  $\beta$ -asarone, while 200 mg/kg body weight/day water in the control group. On the fourth day, the  $\beta$ -asarone in the blood was excluded with the cardio-perfusion method, and the hippocampus, brainstem, cortex and cerebellum in the same regions of the rat brains were harvested on the ice stage. The  $\beta$ -asarone with a purity of 99.55% was prepared.

### Experimental methods

#### The cardio-perfusion method

Thirty minutes after the last intragastric administration, all rats were anesthetized with a single intraperitoneal injection of chloral hydrate solution in a dose of 300 mg/kg body weight. Then the Limbs of the anesthetized rats were fixed on the autopsy tables and the rat hearts were exposed in the thoracic cavities by opening the abdominal cavities which are below the xiphoids. In order to clear away the blood of the rat brain, the normal saline was perfused into the left ventricle and evacuated from the right atrial appendage. The perfusion could be stopped when the rat eyes and claws turned pale.

#### The preparation of GC-MS specimens

The harvested brain tissues were washed with some ice normal saline and weighted after the normal saline was blotted. Then the brain tissues were added methanol in a dose of 20 ml/g in order to be homogenized. The homogenate was centrifuged with a speed of 10000 rpm/min at 4°C for 10 min. One ml supernatant was transferred to 2 ml Eppendorf tube and then evaporated at room temperature. Finally, the evaporated specimens were added with 2 ml ether and filtrated with the millipore filter before the GC-MS analysis.

#### The conditions of GC-MS analysis

Analysis was performed on a shimadzu QP-5000 GC-MS system (shimadzu Corp., Kyoto, Japan). The system was equipped with a DB-1 Quartz capillary column (30 m  $\times$  0.25 mm I.D., 0.25  $\mu$ m film thickness; Agilent J&W). The injector temperature was 250°C and

the interface temperature was 230°C. The column was held 60°C for 10 min, and then raised to 170°C at 7°C/min and to 280°C at 20°C/min. One  $\mu$ l of the specimen mixture was injected in split mode (5:1, v/v), and the helium gas flow rate through the column was 1.3 ml/min. Ions were generated by a 70 kV electron impact, and were recorded at the mass of 208 m/z in the selected ion monitoring mode.

### The investigation of GC-MS analysis conditions

**The precision test:** The same  $\beta$ -asarone aether solution (50 mg/ml) was analyzed continually for five times.

**The stability test:** The  $\beta$ -asarone aether solution (50 mg/ml) was analyzed at 1, 2, 4, 24, 48 and 120 h, respectively.

**The reproducibility test:** Five different  $\beta$ -asarones which were extracted from the same batch of *A. tatarinowii* Schott were prepared and then the five  $\beta$ -asarone aether solutions (50 mg/ml) were analyzed.

### Statistical analyses

Measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ) and compared by analysis of variance and  $p < 0.05$  was considered significantly different. All statistical analyses were performed with version SPSS 13.0 statistical software.

## RESULTS

### The investigation results of GC-MS analysis conditions

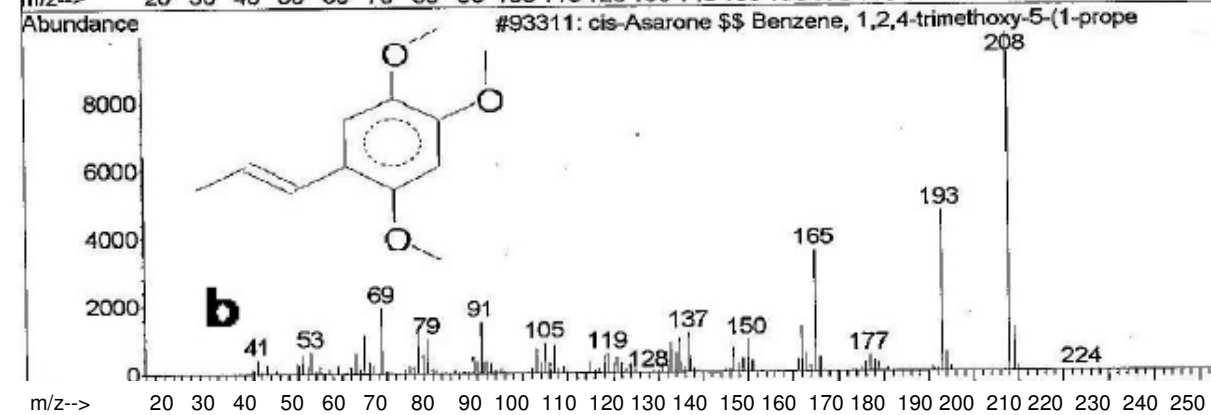
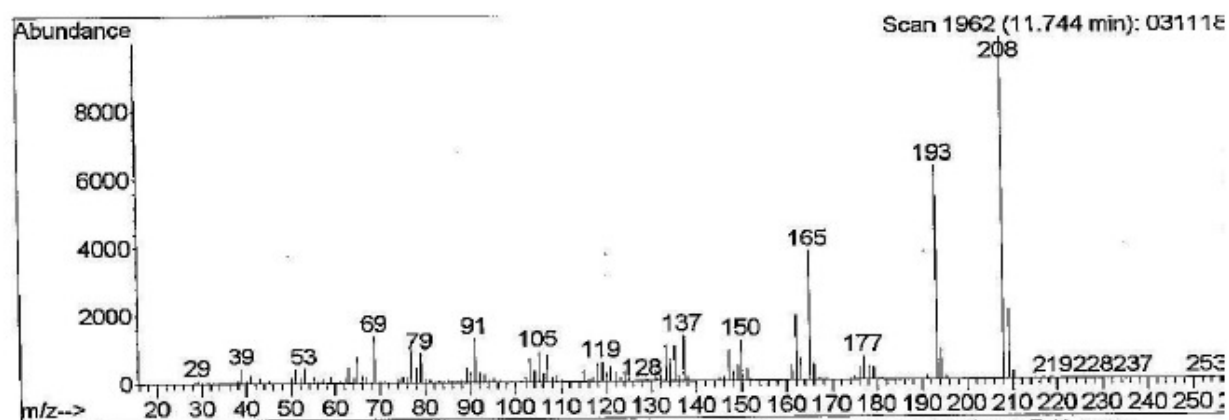
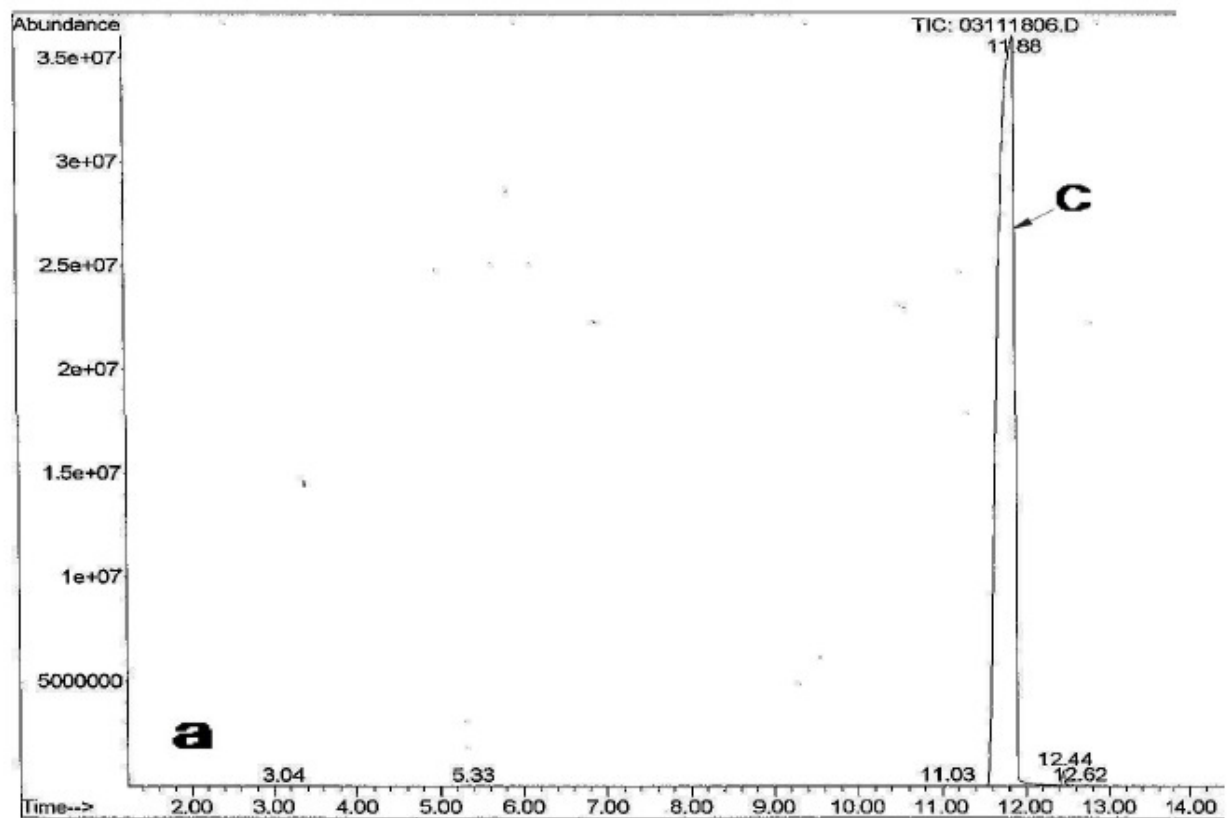
The mean of the relative amount of  $\beta$ -asarone was 99.56% and the relative standard deviation (RSD) was 0.07% in the precision test, while 99.52% and 0.07% in the reproducibility test (Table 1). In the stability test, the mean of the relative amount of  $\beta$ -asarone was 99.53% and the RSD was 0.06% (Table 2).

### The distribution of $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum

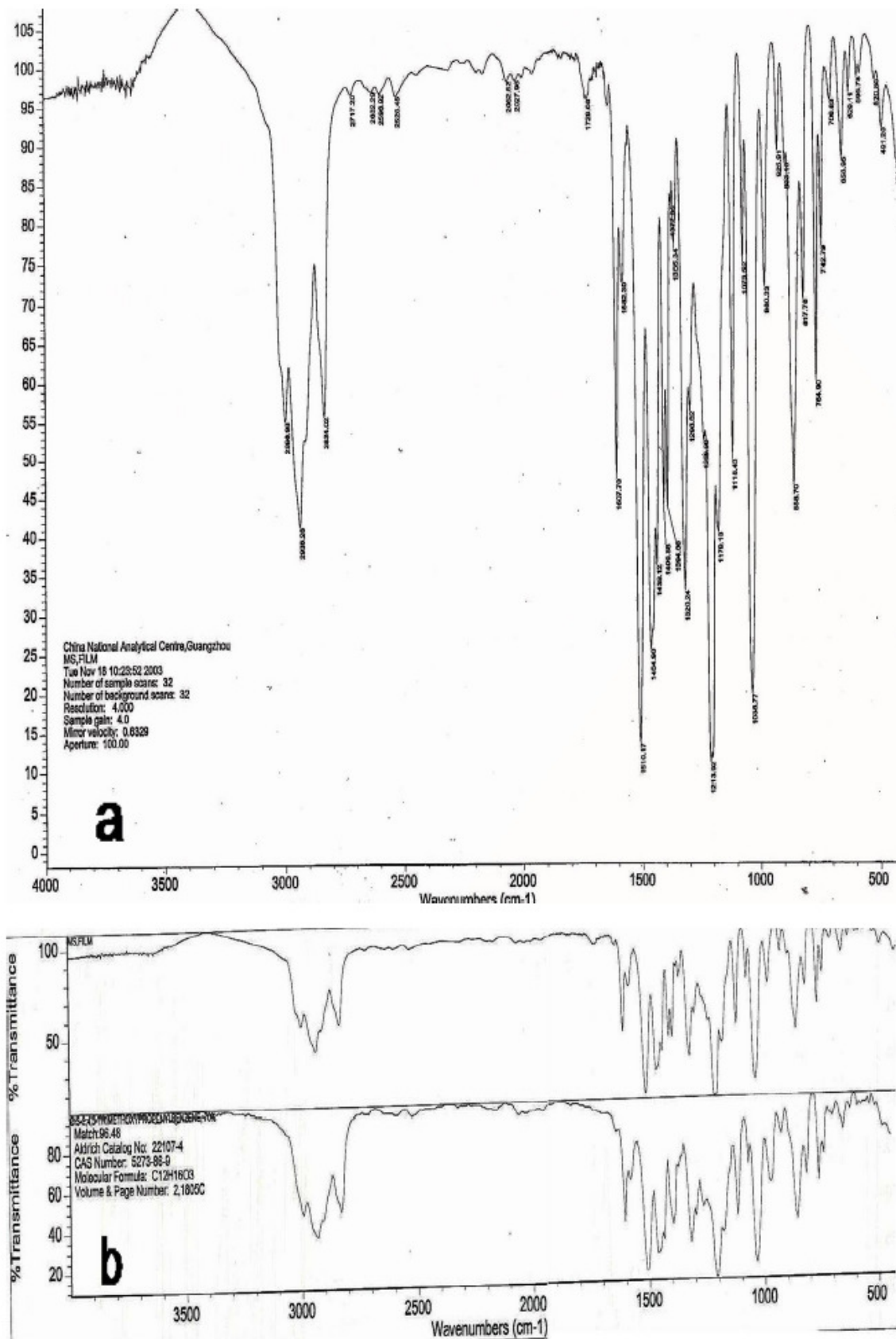
All brain specimens were analyzed under the preceding GC-MS analysis conditions, and peak heights were used to weight the amount of  $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum. The  $\beta$ -asarone could be found in rat hippocampus, brainstem, cortex and cerebellum of the treatment group but not in the blank control group (Figure 4). The peak heights had no significant difference among the hippocampus, brainstem, cortex or cerebellum ( $p > 0.05$ , Table 3).

## DISCUSSION

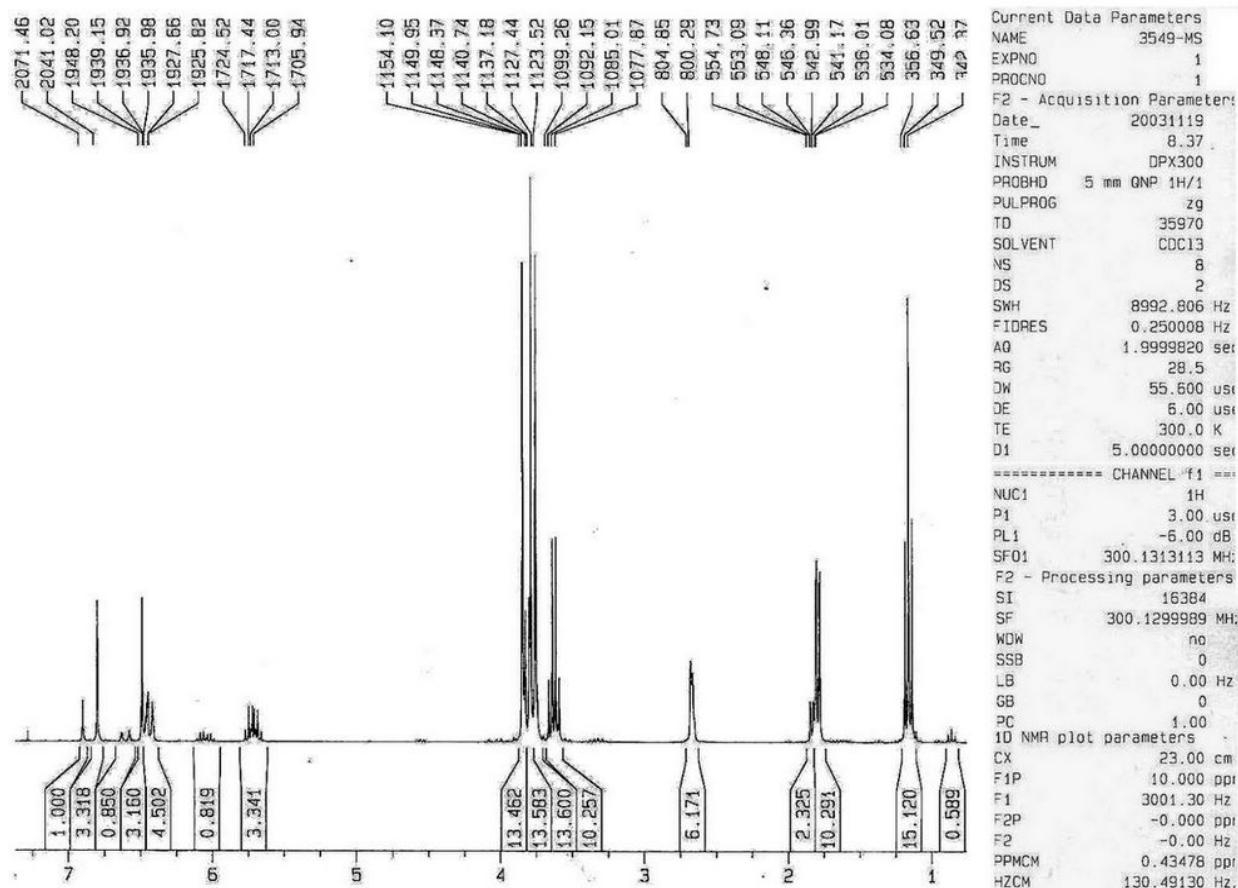
The stability, precision and reproducibility of GC-MS analysis conditions were obtained by the precision test



**Figure 1.** The GC-MS chromatogram of  $\beta$ -asarone (a) and search results of the matches (b). The top peak (c) was  $\beta$ -asarone.



**Figure 2.** The IR chromatogram of  $\beta$ -asarone (a) and search results of the matches (b). Library name: Aldrich FT-IR Collection Edition II, Georgia State forensic drugs.



**Figure 3.** The NMR chromatogram of  $\beta$ -asarone (on the left side) and the parameters of the chromatogram conditions (on the right side).

**Table 1.** The precision test and reproducibility test of GC-MS analysis conditions.

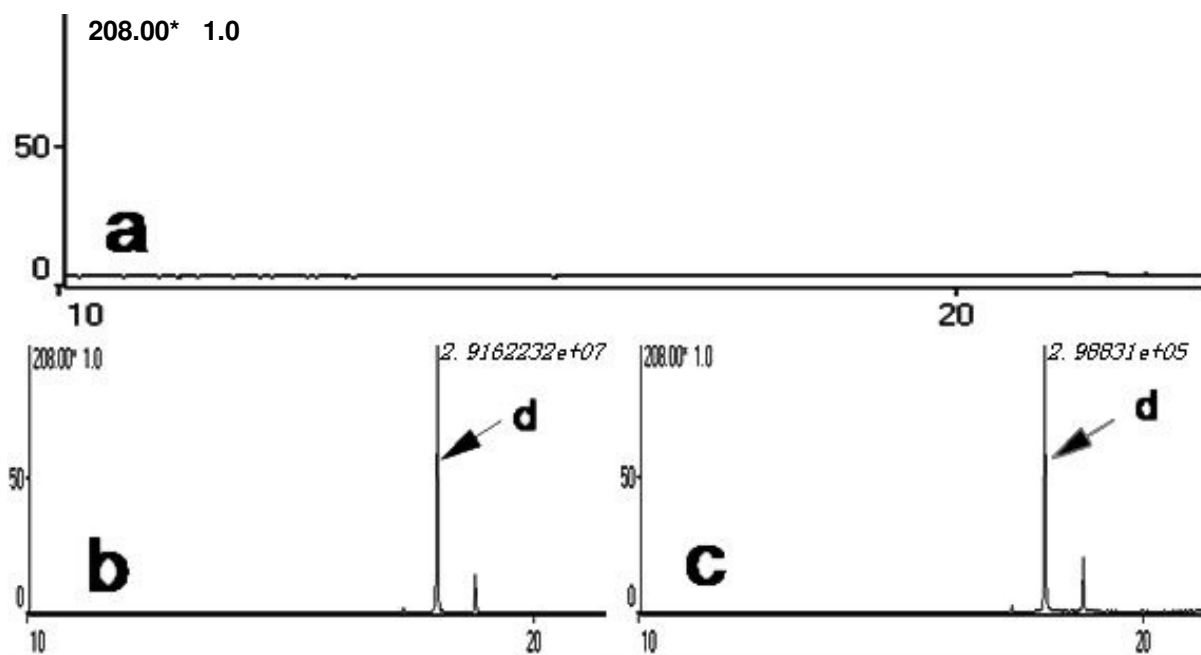
Test type	Precision test					Reproducibility test				
	1	2	3	4	5	1	2	3	4	5
Injection order										
Relative amount (%)	99.67	99.56	99.47	99.58	99.54	99.42	99.55	99.59	99.56	99.48
mean			99.56					99.52		
RSD			0.07					0.07		

The precision of GC-MS analysis Conditions was determined by treating the same  $\beta$ -asarone aether solution for five times in the precision test, and the reproducibility was determined by treating five different  $\beta$ -asarones which were extracted from the same batch of *Acorus tatarinowii* Schott in the reproducibility test. RSD was the written abbreviation of relative standard deviation.

**Table 2.** The stability test of GC-MS analysis conditions.

Test type	Stability test					
	1	2	4	24	48	120
Injection time (h)						
Relative amount (%)	99.43	99.54	99.61	99.56	99.49	99.53
mean				99.53		
RSD				0.06		

The stability of GC-MS analysis Conditions was determined by treating the same  $\beta$ -asarone aether solution at 1, 2, 4, 24, 48 and 120 h, respectively. RSD was the written abbreviation of relative standard deviation.



**Figure 4.** The mass spectrum of  $\beta$ -asarone with GC-MS. a: the mass spectrum of  $\beta$ -asarone in the blank control group; b: the typical mass spectrum of  $\beta$ -asarone solution; c: the typical mass spectrum of  $\beta$ -asarone in the treatment group; d: the peak of  $\beta$ -asarone.

**Table 3.** The distribution of  $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum.

Groups	Peak height			
	Hippocampus	Brainstem	Cortex	Cerebellum
Blank control group	-	-	-	-
Treatment group	261612 $\pm$ 17280	271052 $\pm$ 20108	251162 $\pm$ 16710	271866 $\pm$ 21490

Data are given as mean  $\pm$  SD (n = 8). \*Significant level  $p < 0.05$ . Peak heights were used to weight the amount of  $\beta$ -asarone in rat hippocampus, brainstem, cortex and cerebellum.

and the reproducibility test. This method prompts that the  $\beta$ -asarone can show peak completely within 30 min. In the precision test, the precision of GC-MS system was fine according that the coefficient variation of relative amount and retention time were less than 1 and 0.2%, respectively. In the stability test, the stability of  $\beta$ -asarone solution was good in 120 h according that the coefficient variation of relative amount and retention time were less than 1 and 0.3%, respectively. In the reproducibility test, the reproducibility of  $\beta$ -asarone was fine according that the coefficient variation of relative amount and retention time were less than 1 and 0.3%, respectively.

With the cardio-perfusion method, the effects of  $\beta$ -asarone in brain blood were excluded. The results suggest that  $\beta$ -asarone could be detected in rat hippocampus, brainstem, cortex and cerebellum but the  $\beta$ -asarone amount was less than the previous results (Fang et al., 2002), which indicated the  $\beta$ -asarone in

brain blood had affected the assay of  $\beta$ -asarone in the brain tissues. Meanwhile, we found that distribution of  $\beta$ -asarone among the hippocampus, brainstem, cortex and cerebellum did not have significant differences. We concluded  $\beta$ -asarone could pass through BBB and be widely distributed without target regions in the brain.

The  $\beta$ -asarone has wide pharmacological effects on the CNS. It has been reported that  $\beta$ -asarone could attenuate neuronal apoptosis in rat hippocampus (Liu et al., 2010; Li et al., 2010), and could reduce the toxicity of excitatory amino acids in the epileptic rat brain and increase the expression of c-fos (Fang et al., 2008). Additionally,  $\beta$ -asarone could reduce the injuries of blood vessel endothelium and nerve cells of the cortex (Fu et al., 2008; Chen et al., 2007) and improve the cognitive function of the Beta-amyloid hippocampus injection rats (Geng et al., 2010). The results of this study indicate that  $\beta$ -asarone can be widely distributed in rat hippocampus, brainstem,

cortex and cerebellum without target regions in the brain, which accords with the results that  $\beta$ -asarone, has a wide range of pharmacological effects on CNS.

The BBB plays a very important role in maintaining the normal function of CNS. It can stop some noxious substances of blood going into the CNS. But on the other hand, the BBB increases the difficulties in curing the CNS diseases because it stops about 95% drugs entering the CNS (Hawkins et al., 2005). Only the strong fat-soluble drugs whose molecular weights are less than 400 can go through the BBB (Pardridge et al., 2005). The  $\beta$ -asarone is a strong fat-soluble substance with a small molecular weight (208), which can go through the BBB rapidly. These results suggest that  $\beta$ -asarone might be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease (Liu et al., 2010; Li et al., 2010).

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