A Simple Gas Chromatographic Method for the Simultaneous Determination and Pharmacokinetic Study of Tetramethylpyrazine Phosphate and Borneol in Mouse Plasma and Brain Tissue After Oral Administration of the Fufang Tetramethylpyrazine Phosphate Tablets

Xiao Yan-yu, Ping Qi-neng*, and **Chen Zhi-peng**
Department of Pharmacy, China Pharmaceutical University, 210009 Nanjing, China

Abstract

A rapid, sensitive, and simple gas chromatographic method with flame ionization detection is developed for the simultaneous determination of tetramethylpyrazine phosphate (TMPP) and borneol in mouse plasma and brain tissue. Sample preparations are carried out by deproteinization with an internal standard solution in methanol. The analytes and internal standard (dimethyl sulfoxide) are well-separated on an HP-5 MS capillary column. The analytical curves are linear over a wide concentration range of 0.02–40 µg/mL for both TMPP and borneol in plasma and brain tissue, with the intra- and inter-day precision (the relative standard deviation values) at less than 15%. TMPP and borneol are both stable under different conditions. The method described is successfully applied to the pharmacokinetic study of mouse plasma and brain tissue after oral administration of the Fufang TMPP and TMPP tablets to mice.

Introduction

Tetramethylpyrazine phosphate (TMPP) (Figure 1A) has been widely used in China for the treatment of cardiovascular and cerebrovascular disorders (1–3). It has been found to block calcium channels, reduce the bioactivity of platelets and platelet aggregation, inhibit free radicals, increase cerebral blood flow (4–6), and improve blood viscosity (7). However, TMPP possesses a low oral bioavailability (10–30%) after oral administration, due to hepatic first pass metabolism (8).

Borneol (Figure 1B and 1C), a monoterpenoid alcohol, is isolated from *Fructus amoni*. Recently, it has been reported that borneol can increase the oral bioavailability of TMPP and enhance its distribution in brain tissue. To increase oral bioavailability of TMPP and to enhance its distribution in brain tissue, the Fufang TMPP tablets containing TMPP and borneol were developed for the treatment of cerebral thrombosis. Therefore, it was necessary to establish a sensitive and simple method to simultaneously determinate TMPP and borneol in biological fluids.

To date, several analytical methods have been developed for the determination of tetramethylpyrazine (TMP) and borneol in biological fluids. Peng et al. (9) determined the concentrations of TMP and its metabolite in dog plasma using a liquid chromatographic–mass spectrometric (LC–MS) method. Tung-Hu et al. (10) researched the pharmacokinetics of TMP in rat blood and brain using microdialysis. Liang et al. (11–12) measured the pharmacokinetics of TMP in rat blood and regional brain tissue by high-performance LC with UV detection, and also studied the pharmacokinetics of borneol in rat blood and brain tissue by gas chromatography (GC). Guo et al. (13) studied the simultaneous determination of TMP and bornoel in human plasma, administrated with Suxiao Jiuxin Wan. But most of those methods require complicated sample preparation procedures involving basification of the plasma sample, extraction, acidification, preconcentration, and reconstitution of the analytes. These procedures are rather time-consuming and more complex than the procedure described in this paper. With this method, samples using dimethyl sulfoxide as an internal standard could be applied to GC with flame ionization detection (FID) with simple deproteinization and without multiple pretreatment steps. The present method was also successfully applied to the pharmacokinetic study of mouse plasma and brain tissue after oral administration of the Fufang TMPP tablets and TMPP tablets to mice.

* Author to whom correspondence should be addressed: email pingqn@cpu.edu.cn.

Experimental

Materials

Tetramethylpyrazine phosphate (TMPP, 99.8% purity) was purchased from Beijing Yanjing Pharmaceutical Factory (Beijing, China). Borneol (99.4% purity) and dimethyl sulfoxide (internal standard, I.S.) was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). The other chemical reagents were of analytical grade or better.

The reference formulation consisted of TMPP tablets purchased from Beijing Yanjing Pharmaceutical Factory (Beijing, China). Each tablet contains 50 mg of TMPP. The Fufang TMPP tablets were prepared in our laboratory. Each tablet contains 50 mg of TMPP and 40 mg of borneol.

Instrument and chromatographic conditions

The GC–FID system consisted of an HP 4890 gas chromatographic system, an FID (Agilent 4890, Palo Alto, CA), and Agilent Chemstation software for data analysis. Separation of TMPP, borneol, and I.S. from endogenous substances was performed on an HP-5 MS capillary column (30.0 m × 250 µm, 0.25 µm). Nitrogen, at a flow rate of 25 mL/min, was used as the carrier gas. The split mode with a split ratio of 10:1 was used in the study. Upon the injection of the sample to the chromatograph, firstly, the oven temperature was 100°C for 1 min, then increased from 100°C to 160°C at a rate of 30°C/min, then to 200°C at a rate of 5°C/min, and was thereafter maintained at 200°C for 4 min. The injection port temperature was maintained at 180°C; detector temperature was maintained at 210°C. Samples of 1 µL were injected manually.

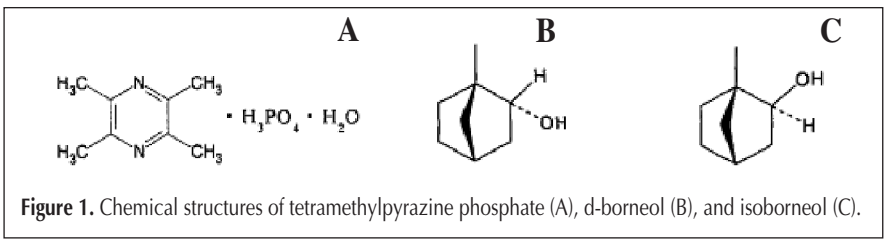

Figure 1. Chemical structures of tetramethylpyrazine phosphate (A), d-borneol (B), and isoborneol (C).

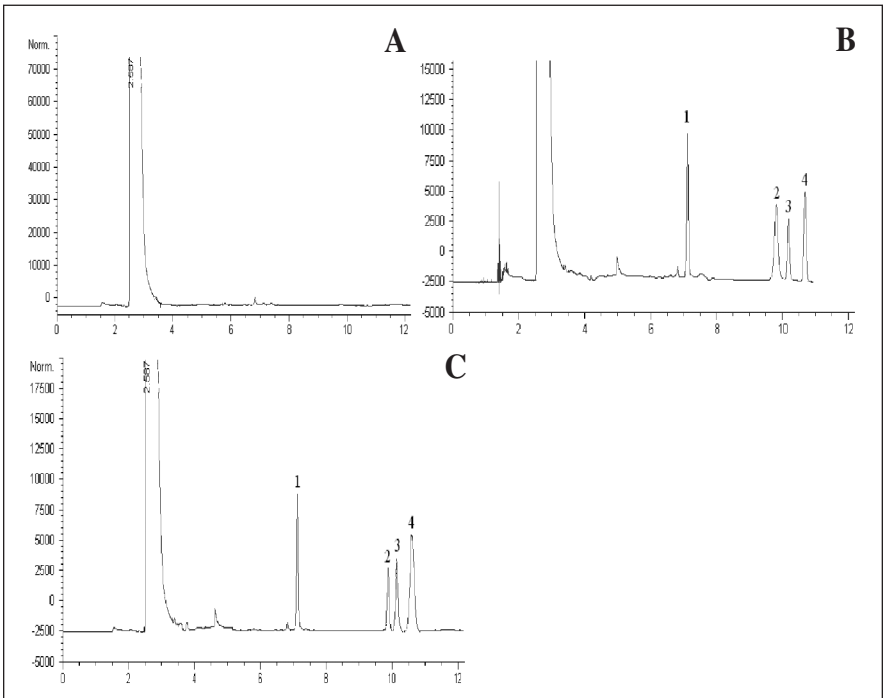

Figure 2. Typical gas chromatograms of blank mouse plasma (A), blank mouse plasma spiked with 3 µg/mL TMPP, 1 µg/mL borneol, and I.S. (B), and plasma sample at 5 min after oral administration of the Fufang tablets (TMPP, equivalent to 37.5 mg/kg and borneol, equivalent to 30 mg/kg) (C). TMPP, 1; I.S., 2; isoborneol, 3; and d-borneol, 4.

Preparation of stock solution and calibration standard solutions

The reference standards (TMPP and borneol, 10.0 mg each) were accurately weighed into a 10-mL volumetric flask and dissolved in methanol to make a stock solution. A series of standard solutions were obtained by further dilution of the stock solutions with methanol. The I.S. solution was prepared in methanol at a concentration of 1 µL/mL. All solutions were stored at 4ºC. Two microliters of the standard solution were transferred into 98 µL of blank mouse plasma to obtain a calibration standard concentration of 0.02, 0.1, 0.2, 1.6, 3, 12, and 40 µg/mL of both TMPP and borneol. Four microliters of the standard solution were added to 196 µL of blank brain homogenate to obtain the same calibration standard concentrations of both TMPP and borneol as that in plasma. Quality control (QC) samples containing both TMPP and borneol of 0.02, 3, and 40 µg/mL were prepared in the same way.

Sample preparation

One hundred microliters of each plasma sample were transferred to a 1.5-mL polyethylene centrifuge tube. Ten microliters of phosphate buffer saline (pH 8.0) were added to each plasma sample and vortex-mixed for approximately 30 s. One hundred fifty microliters of I.S. solution were added and vortexed for 1 min. The denatured protein precipitate was separated by centrifugation at 12000 rpm for 10 min. One microliter of supernatant was directly injected into the GC–FID apparatus for analysis.

Two hundred microliters of each brain homogenate sample were transferred to a 1.5-mL polyethylene centrifuge tube. Twenty microliters of phosphate buffer saline (pH 8.0) were added to each plasma sample and vortex-mixed for approximately 30 s. One hundred fifty microliters of internal standard solution were added and vortexed for 1 min. The denatured protein precipitate was separated by

centrifugation at 12000 rpm for 10 min. One microliter of supernatant was directly injected into the GC–FID apparatus for analysis.

Validation of assay method

The selectivity of the method was investigated by comparing the FID chromatograms of blank plasma or blank brain homogenate, standard plasma sample or standard brain homogenate sample spiked with dimethyl sulfoxide, TMPP and borneol, and plasma sample or brain homogenate sample at 5 min after oral administration of the Fufang TMPP tablets (TMPP, equivalent to 37.5 mg/kg and borneol, equivalent to 30 mg/kg). The linearity of each calibration curve was determined by plotting the peak-area ratio (y) of the analyte to I.S. versus the nominal concentration (x) of either TMPP or borneol.

The intraday accuracy and precision of TMPP and borneol determinations were analyzed by performing the determinations five times in the same day at concentrations of 0.02, 3, and 40 µg/mL. The interday accuracy and precision of TMPP and borneol determinations were also analyzed, by repeating the determinations five times on three different days. Assay precision was expressed as the relative standard deviation (RSD) (coefficient of variation). Accuracy (expressed as percent of nominal values) was determined by comparing the concentration calculated from the calibration curve to the known concentration.

The recoveries of TMPP and borneol were determined at low (0.02 µg/mL), medium (3 µg/mL), and high (40 µg/mL) concentrations by comparing the peak areas from plasma samples or brain homogenate samples spiked before extraction with those from standard solutions at the same levels (three samples for each concentration level).

Sample stability was determined by analyzing QC samples after three freeze-thaw cycles and exposed to ambient temperature or –20°C over a time period of 24 h.

Application of the assay

The animal experiments gained the China ethic committee's approval. One

Table I. Intra-Day (n = 5) and Inter-Day (n = 3) Precision and Accuracy of the Determinations of TMPP and Borneol in Plasma Samples

		Intra-day (n = 5)			Inter-day (n = 3)		
Analytes	Spiked value (µg/mL)	Concentration found (mean ± SD) (µg/mL)	Precision* (%)	Accuracy† (%)	Concentration found (mean ± SD) (µg/mL)	Precision* (%)	Accuracy† (%)
TMPP	0.02	0.019 ± 0.0011	5.79	95.49	0.019 ± 0.0016	8.33	93.48
	3.0	2.98 ± 0.064	2.14	99.38	2.96 ± 0.13	4.30	98.64
	40.0	40.21 ± 0.46	1.14	100.52	39.25 ± 1.04	2.66	98.12
Borneol	0.02	0.019 ± 0.0011	5.79	96.54	0.019 ± 0.0021	11.05	97.66
	3.0	2.79 ± 0.15	5.34	93.12	2.83 ± 0.18	6.42	94.46
	40.0	37.94 ± 1.18	3.11	94.86	36.94 ± 1.60	4.33	92.34

* ± Expressed as RSD%: (S.D. / mean) × 100.
† Calculated as (%): (mean concentration found / spiked value) × 100.

Table II. Intra-Day (n = 5) and Inter-Day (n = 3) Precision and Accuracy of the Determinations of TMPP and Borneol in Brain Tissue Samples

		Intra-day (n = 6)			Inter-day (n = 5)		
Analytes	Spiked value (µg/g)	Concentration found (mean ± SD) (µg/g)	Precision* (%)	Accuracy† (%)	Concentration found (mean ± SD) (µg/g)	Precision* (%)	Accuracy† (%)
TMPP	0.02	0.019 ± 0.0019	10.00	96.42	0.019 ± 0.0027	13.89	97.21
	3.0	2.79 ± 0.086	3.09	93.13	2.96 ± 0.18	6.20	98.75
	40.0	40.21 ± 0.54	1.34	100.52	39.45 ± 0.90	2.28	98.63
Borneol	0.02	0.019 ± 0.0015	7.89	96.44	0.019 ± 0.0018	9.47	97.24
	3.0	2.96 ± 0.10	3.42	98.71	2.86 ± 0.20	6.84	95.37
	40.0	39.90 ± 1.42	3.56	99.75	38.34 ± 2.15	5.61	95.86

* ± Expressed as R.S.D.%: (S.D. / mean) × 100.
† Calculated as (%): (mean concentration found / spiked value) × 100.

Table III. Recoveries of TMPP and Borneol in Plasma Samples and Brain Tissue Samples (n = 3)

		Plasma			Brain tissue		
Analytes	Spiked value (µg/mL) or (µg/g))	Concentration found (mean ± SD) (µg/mL)	Recovery (%)	RSD (%)	Concentration found (mean ± SD) (µg/g)	Recovery (%)	RSD (%)
TMPP	0.02	0.019 ± 0.0006	96.45	3.16	0.019 ± 0.0009	95.32	4.74
	3.0	2.96 ± 0.12	98.54	4.11	2.94 ± 0.11	98.12	3.92
	40.0	39.65 ± 0.47	99.13	1.19	39.50 ± 0.76	98.76	1.94
Borneol	0.02	0.019 ± 0.001	96.32	5.31	0.016 ± 0.001	80.78	7.93
	3.0	2.95 ± 0.094	98.41	3.22	2.41 ± 0.16	80.41	6.72
	40.0	39.35 ± 1.41	98.37	3.55	31.82 ± 1.32	81.54	4.08

hundred and twenty male mice (18–22 g), divided randomly into two groups, were fasted for 24 h with free access to water. The Fufang TMPP tablets were ground to powder and some powder equivalent to 37.5 mg/kg TMPP and 30 mg/kg borneol was suspended in 0.5 mL of 0.5% CMC–Na solution, then were orally administered to one group of mice. The TMPP tablets were also ground to powder and some powder equivalent to 37.5 mg/kg TMPP was suspended in 0.5 mL of 0.5% CMC–Na solution, then were orally administrated to another group of mice. After oral administration, mice were killed by decapitation after taking blood by removing an eyeball at time intervals of 2, 5, 10, 15, 20, 30, 40, 60, 90, and 120 min, respectively, and the cerebra were removed and weighted. At each sampling time, six mice were killed and sampled.

Plasma samples were collected at several intervals after centrifugation at 4000 rpm for 5 min and then stored immediately at –20°C. The brain regions were homogenized with physiological saline (2 mL/1g tissue) using a tissue homogenizer (Shanghai, China) and then frozen at –20°C.

Peak concentration (C_{max}) and peak times (T_{max}) of TMPP and borneol were derived directly from the experimental points. The other pharmacokinetic parameters were fitted using the 3P97 pharmacokinetics program (the Section of Mathematical Pharmacology of Chinese Mathematical Pharmacological Society).

Table IV. Stability of TMPP in Mouse Plasma Under Different Storage Conditions

Storage conditions	Concentration added (μg/mL)	Concentration found (mean ± SD, μg/mL)	Remaining percentage (%)
Freeze-thaw	0.02	0.019 ± 0.0019	97.66
	3.0	2.82 ± 0.049	95.67
	40.0	38.42 ± 0.38	96.05
At room temperature, 24 h	0.02	0.019 ± 0.0017	96.54
	3.0	2.89 ± 0.13	96.33
	40.0	40.09 ± 1.24	100.22
At –20°C, 24 h	0.02	0.019 ± 0.0015	95.48
	3.0	2.96 ± 0.13	98.67
	40.0	38.96 ± 1.24	98.14

Table V. Stability of Borneol in Mouse Plasma Under Different Storage Conditions

Storage conditions	Concentration added (μg/mL)	Concentration found (mean ± SD, μg/mL)	Remaining percentage (%)
Freeze-thaw	0.02	0.019 ± 0.0015	98.26
	3.0	2.91 ± 0.14	97.33
	40.0	38.10 ± 0.92	96.25
At room temperature, 24 h	0.02	0.019 ± 0.0016	96.34
	3.0	3.08 ± 0.58	102.67
	40.0	39.74 ± 1.95	99.35
At –20°C, 24 h	0.02	0.019 ± 0.0017	96.87
	3.0	2.82 ± 0.13	95.67
	40.0	39.32 ± 1.24	98.37

Table VI. Stability of TMPP in Mouse Brain Tissue Under Different Storage Conditions

Storage conditions	Concentration added (μg/mL)	Concentration found (mean ± SD, μg/g)	Remaining percentage (%)
Freeze-thaw	0.02	0.019 ± 0.0018	96.18
	3.0	2.94 ± 0.12	98.26
	40.0	39.58 ± 1.26	98.95
At room temperature, 24 h	0.02	0.019 ± 0.0017	97.14
	3.0	2.92 ± 0.18	97.33
	40.0	39.56 ± 2.04	98.92
At –20°C, 24 h	0.02	0.021 ± 0.0015	105.12
	3.0	2.90 ± 0.42	96.67
	40.0	39.86 ± 3.54	99.65

Results and Discussion

Specificity

The chromatograms (Figure 2) were free of interference from other compounds after precipitation of the protein from tissue homogenates and plasma, which showed the retention times of 7.1 and 9.8 min for TMPP and dimethyl sulfoxide, respectively; however, two peaks were detected at approximately 10.0 min and 10.5 min, because borneol contained isobrneol and d-borneol and the area of the two peaks was used for pharmacokinetic analysis. In conclusion, these observations indicate that the specificity of the assay is adequate.

Sensitivity

Under the experimental conditions used, the lower limit of detection (LOD) of TMPP in both plasma and brain tissue was 5 ng/mL at signal-to-noise ratio of 3, and for borneol in plasma and brain tissue, 2 ng/mL and 5 ng/mL, respectively. The lower limit of quantitation (LOQ) of TMPP in both plasma

and brain tissue was found to be 20 ng/mL, and for borneol in both plasma and brain tissue, 20 ng/mL.

Linearity

The calibration curves for both TMPP and borneol in plasma and brain tissue were linear in the range of 0.02–40 µg/mL. Using the linear least squares regression, the calibration line of TMPP was $y = 0.0021x + 0.013$ with $r^2 = 0.9952$ in plasma and $y = 0.0034x - 0.0018$ with $r^2 = 0.9964$ in brain tissue. For borneol, the calibration line was $y = 0.001x - 0.0097$ with $r^2 = 0.9986$ in plasma and $y = 0.0014x + 0.0028$ with $r^2 = 0.9802$ in brain tissue.

Precision and accuracy

The summaries of intra- and inter-day precision/accuracy at low, medium, and high concentrations of TMPP and borneol in plasma and brain tissue are listed in Tables I and II. The intra- and inter-day precision of both TMPP and borneol were within 10.0% in plasma and brain tissue. The intra- and inter-day accuracy ranged from 93.13% to 100.52% in plasma and brain tissue.

These results indicate that the accuracy and precision of the current assay are within the recommendations for the assay validation by "Guidance for Industry: Bioanalytical Method Validation (FDA, May 2001)", which was required to yield a precision of less than 20% (RSD) and an accuracy between 80% and 120% of the theoretical value and the reproducibility of the assay is adequate.

Extraction recovery

The extraction recoveries of both TMPP and borneol in plasma samples or brain homogenate samples were determined by comparing peak areas from plasma samples or brain homogenate samples spiked before extraction with those from standard solutions at the same levels. The summaries of the mean recoveries are listed in Table III. The results showed that the extraction recoveries of TMPP ranged from 96.45% to 99.13% in plasma and 95.32% to 98.76% in brain tissue, and from 96.32% to 98.41% for borneol in plasma and 80.41% to 81.54% in brain tissue. The mean extraction recoveries of borneol in brain tissue were approximately 80%, independent of concentrations, and it was considered that borneol could combined with the liposolubility constituent of brain tissue, which lead to insufficient extraction and low recoveries.

Stability

The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior to the analysis. The summaries are listed in Tables IV to VII. The study results showed no significant degration of either TMPP or borneol in the plasma or brain tissue homogenate samples under every experimental condition. Mean TMPP and borneol concentrations ranged from 95.48–102.67% in plasma; however, concentrations in brain homogenate sample were 95.72–105.12%.

Application of the developed GC–FID method

The GC–FID method developed has also been successfully used for the pharmacokinetic study of mice following oral administration of the Fufang TMPP tablets equivalent to 37.5 mg/kg TMPP and 30 mg/kg borneol, and the TMPP tablets equivalent to 37.5 mg/kg TMPP. The mean plasma and brain tissue concentration-time profiles of both TMPP and borneol are shown in Figures 3 and 4, respectively. After oral administration of TMPP tablets, the C_{max} of TMPP was 9.09 ± 1.45 µg/mL and the $AUC_{0–t_{120}}$ was 131.46 ± 4.81 µg/mL/min. In brain tissue, C_{max} of TMPP was 2.83 ± 0.74 µg/g and the $AUC_{0–t_{120}}$ was 103.54 ± 2.35 µg/g/min. However, after oral administration of the Fufang TMPP tablets, the C_{max} of TMPP in plasma was 15.26 ± 2.97 µg/mL and the $AUC_{0–t_{120}}$ was 289.82 ± 66.81 µg/mL/min, which was approximately 2.21 times compared with that in oral administration of TMPP tablets. C_{max} of TMPP in brain tissue was 4.38 ± 1.24 µg/g and the $AUC_{0–t_{120}}$ was 168.15 ± 29.35 µg/g/min, which was approximately 1.62 times compared with that of oral administration of TMPP tablets. At the same time, after oral administration of the Fufang TMPP tablets, C_{max} of borneol in plasma was 2.64 ± 0.054 µg/mL and the $AUC_{0–t_{120}}$ was 67.15 ± 5.88 µg/mL/min. C_{max} of borneol in brain tissue was 4.87 ± 0.12 ug/g and the $AUC_{0–t_{120}}$ was 101.22 ± 4.69 µg/g/min. From these data, it was obvious that borneol did increase the oral bioavailability of TMPP and enhance its distribution in brain tissue.

Conclusions

Sample preparation plays a key role in the determination of drugs in biological samples. At the beginning of this work, liquid–liquid extraction was tried and then the organic solvent was dried, but we found that the drying process in

Table VII. Stability of Borneol in Mouse Brain Tissue Under Different Storage Conditions

Storage conditions	Concentration added (µg/mL)	Concentration found (mean ± SD, µg/g)	Remaining percentage (%)
Freeze-thaw	0.02	0.019 ± 0.0017	96.29
	3.0	3.01 ± 0.38	100.33
	40.0	39.14 ± 2.48	97.86
At room temperature, 24 h	0.02	0.019 ± 0.0017	95.72
	3.0	2.94 ± 0.58	98.37
	40.0	39.04 ± 2.36	97.68
At –20°C, 24 h	0.02	0.020 ± 0.0012	100.09
	3.0	2.97 ± 0.29	99.14
	40.0	39.02 ± 2.98	97.56

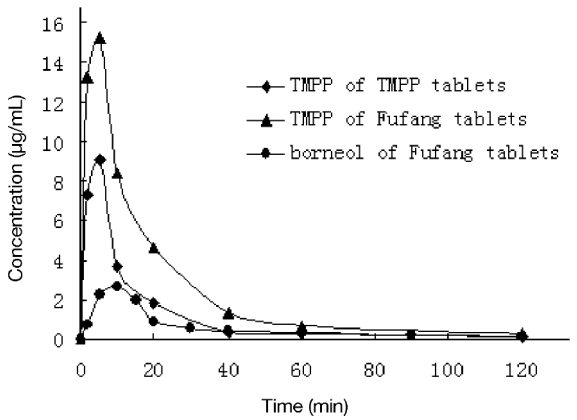

Figure 3. Mean plasma concentration-time profiles of TMPP and borneol in mice after oral administration of the Fufang tablets equivalent to 37.5 mg/kg TMPP and 30 mg/kg borneol, and the TMPP tablets equivalent to 37.5 mg/kg TMPP (n = 6).

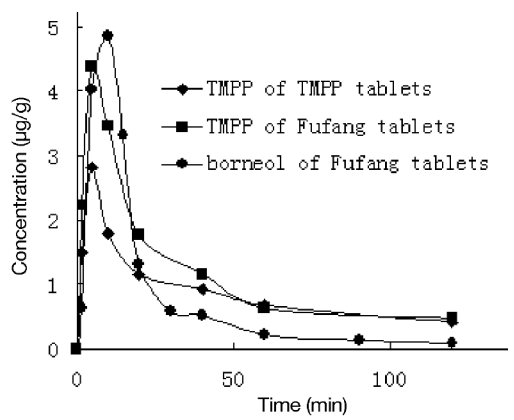

Figure 4. Mean brain concentration-time profiles of TMPP and borneol in mice after oral administration of the Fufang tablets equivalent to 37.5 mg/kg TMPP and 30 mg/kg borneol, and the TMPP tablets equivalent to 37.5 mg/kg TMPP (n = 6).

liquid–liquid extraction caused a significant loss of the analytes due to their volatility. After several trials, a protein precipitation procedure was adopted and proved to be simple and reliable for the sample preparation in this work. Methanol rather than acetonitrile or alcohol was selected as the protein-precipitating solvent for producing the expected peak shapes of analytes. For the permanent recovery, phosphate buffer saline (pH 8.0) was used. The I.S. is necessary for the determination of analytes in biological samples using GC. In the initial stages of our work, several compounds were tried and finally dimethyl sulfoxide was found to be optimal for our work.

A rapid, sensitive, and simple GC method has been developed using FID for the simultaneous analysis of TMPP and borneol in mouse plasma and brain tissue. Sample preparation involves protein precipitation using methanol and no evaporation step is required. This method has no significant loss of resolution. Moreover, due to low LOQ (20 ng/mL), good accuracy, and precision, this method is suitable for pharmacokinetic studies of the preparations containing TMPP and borneol in mice.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 30430790).

References

1. S.K. Guo, K.J. Chen, Z.H. Qian, W.L. Weng, and M.Y. Qian. Tetramethylpyrazine in the treatment of cardiovascular and cerebrovascular diseases. *Planta Med.* **47:** 89 (1983).
2. P.K.T. Pang, J.J.Shan, and K.W. Chiu. Tetramethylpyrazine, a calcium antagonist. *Planta Med.* **62:** 431–35 (1996).
3. M.C. Sutter and Y.X. Wang. Recent cardiovascular drugs from Chinese medicinal plants. *Cardiovasc. Res.* **27:** 1891–1901 (1993).
4. S.Y. Liu, and D.M. Sylvester. Antiplatelet activity of tetramethylpyrazine. *Thromb. Res.* **75:** 51–62 (1994).
5. F. Liao. Herbs of activating blood circulation to remove blood stasis. Clin. Hemorheol. *Microcirc.* **23:** 127–131 (2000).
6. L.Y. Zou, X.M. Hao, G.Q. Zhang, M. Zhang, J.H. Guo, and T.F. Liu. Effect of tetramethyl pyrazine on L-type calcium channel in rat ventricular myocytes. *Can. J. Physiol. Pharmacol.* **79:** 621–626 (2001).
7. H. Watanabe. Candidates for cognitive enhancer extracted from medicinal plants: paeoniflorin and tetramethylpyrazine. *Behav. Brain. Res.* **83:** 135–141 (1997).
8. W. Cai, S.N. Dong, and Y.Q. Lou. HPLC determination of tetramethylpyrazine in human serum and its pharmacokinetic parameters. *Acta Pharm. Sinica.* **12:** 881–886 (1989).
9. W. Peng, J. Xin, Q. Meiling, and F. Lin. Liquid chromatography–mass spectrometry method for determination of tetramethylpyrazine and its metabolite in dog plasma. *J. Chromatogr. B Biomed. Sci. Appl.* **813:** 263–268 (2004).
10. T.H. Tsai and C.C. Liang. Pharmacokinetics of tetramethylpyrazine in rat blood and brain using microdialysis. *Int. J. Pharm.* **216:** 61–66 (2001).
11. C.C. Liang, C.Y. Hong, C.F. Chen, and T.H. Tsai. Measurement and pharmacokinetic study of tetramethylpyrazine in rat blood and its regional brain tissue by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **724:** 303–309 (1999).
12. M.R. Liang, Q.T. Liu, T.L. Huang, Y.Q. Zhang, and W.P. Ou. The pharmacokinetic characteristics of borneol in serum and brain tissue of rats. T*raditional Chinese Drug Research & Clinical Pharmacology* **4:** 38–40 (1993).
13. J. Guo, X. Huang, L.L. Wang, H. Meng, L. Zhang, and P. Ren. Synchro-determination of borneol and tetramethyl pyrazine in plasma by GC-FID for volunteers administrated with Suxiao Jiuxin Wan. *Chinese Traditional and Herbal Drugs* **34:** 730-732 (2003).

Manuscript received October 13, 2006;
Revision received May 3, 2007.