Simultaneous Determination of Cinnamaldehyde, Eugenol and Paeonol in Traditional Chinese Medicinal Preparations by Capillary GC-FID

Bing-Sheng YU,* Shu-Guang LAI, and Qiao-Lin TAN

Department of Chemistry, Zhanjiang Normal College; Zhanjiang 524048, China.
Received June 9, 2005; accepted September 8, 2005

A capillary GC method was established for simultaneous determination of cinnamaldehyde (CNMD), eugenol (EL) and paeonol (PL) in two traditional Chinese herbal medicinal preparations, Weitongding tablet (WTDT) and Guifu Dihuang pill (GDHP). The assays were based on a programmed temperature GC in a 30 m×0.53 mm capillary column with nitrogen as carrier and FID detector. Good linearities were obtained over ranges of 0.45—452 mg/l CNMD, 0.31—625 mg/l EL and 0.30—610 mg/l PL, respectively. The spike recoveries were within 84—111%.

Key words GC; cinnamaldehyde; eugenol; paeonol; traditional Chinese medicinal preparation

Mudanpi (Cortex Moutan, Cortex Paeonia), Dingxiang (Eugenia caryophyllata Thunb.), and Rougui (Cortex Cinnamomi) or Guizhi (Ramulus Cinnamomi) are widely used in traditional Chinese medicines and pharmaceutical preparations.[1)] Paeonol (PL) is the main active component in Cortex Moutan, and cinnamicaldehyde (CNMD) is in Cortex (or Ramulus) Cinnamomi. As an intrinsic component of Eugenia caryphyllsta Thunb., eugenol (EL) also occurs in many herbal species, such as Rosa Rugosa Thunb., Murraya paniculata (L.) Jack., and Gaoliangjiang (Rhizoma Alpiniae Officinarum). Thus, qualitative identification or quantitative measurement of the active components are the primary way of authentication of those herbal medicine materials or related preparations.[1)] The versatile biomedical effects of CNMD, EL and PL have been intensively investigated, for example, anti-aggregation of platelet,[2)] antimutagenic[3,4)] and bacteriocidal[5—8)] effects. A protective effect on ischemia and the antilipid peroxidation effect of PL were reported.[9)]

Guifu Dihuang Pill (GDHP) is a common herbal medicinal preparation used to treat asthenia, night sweat and spermatorrhea. This pill preparation is made from 8 herbal medicines, including Rougui (Cortex Cinnamomi), Fuzhi (Radic Aconiti Lateralis Preparata) and Mudanpi (Cortex Paeonia).[1)] Its quality control is based on TLC procedures for qualitative identification of PL and CNMD, and quantitative estimation of ursolic acid.[1)] As a new Chinese traditional patent medicine, the Weitongding tablet (WTDT) is usually used to treat gastritis, gastric malaise due to depression, and gastralgia due to indigestion or gastric cold.[10)] This tablet preparation is made from more than 10 herbal medicines, including Rougui (Cortex Cinnamomi), Dingxiang (Eugenia caryophyllata Thunb.), Honghua (Flos Carthami), and Gaoliangjiang (Rhizoma Alpiniae Officinarum). However, WTDT is not included in the updated Chinese Pharmacopoeia,[1)] its quality control procedures refer to only a simple description about dosage form, appearance and properties, without any qualitative identification or quantitative measurement of active components.[10)] Therefore, it is essential for quality control of the tablet that a reliable and simple method of measuring the related active components be developed.

Determination of CNMD, EL and PL in herbal medicinal materials, pharmaceutical and biomedical samples mainly relies on chromatography techniques, especially HPLC[11—19)] and GC,[20—32)] and TLC is usually used to identify medicinal materials.[1)] Zhou and coworkers,[32)] who reviewed the methodologies for determination of paeonol in traditional Chinese medicines and related medicinal preparations, suggested that capillary GC would be the best choice of paeonol measurement. In this paper, a programmed temperature GC method was established and successfully used to simultaneously determine CNMD, EL and PL in Guifu Dihuang pill and Weitongding tablet.

Experimental

Reagents and Materials Standards of CNMD, EL and PL were purchased from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Other reagents were of analytical grade or better from commercial sources. Double distilled water was used throughout.

Stock solutions of 452.0 mg/l CNMD, 625.0 mg/l EL and 610.0 mg/l PL were prepared with methanol, respectively, and diluted with the same solvent when necessary.

Apparatus Gas chromatographic separation was carried out using a GC-14B gas chromatograph system (Shimadzu, Japan), with nitrogen as carrier gas and a hydrogen FID detector. A CLASS-GC10 GC workstation (Shimadzu, Japan) was used to control the GC system and manipulate the chromatograms. A DB-17 capillary column (30 m×0.53 mm, 1.0 μm film, J&W Scientific, CA, U.S.A.) was used for separation.

An SK3300LH ultrasonic generator (59 kHz, 160 W) from the Shanghai Kudos Ultrasonic Instruments Co., Ltd. (Shanghai, China) was used to extract the objective compounds in the medicinal samples.

Conditions for Chromatographic Separation The carrier gas was at a flow-rate of 60 ml/min, and hydrogen at a flow-rate of 55 ml/min with air at 500 ml/min. GC separation was carried out in a programmed temperature mode. The temperatures of inject port and FID detector were 220 °C and 250 °C, respectively. Five microliters of standard or sample solution was injected in a splitless mode.

Experimental Procedure The chromatograph system was equilibrated by the carrier gas until the same retention times and peak areas of repetitive injections of standard solution (not less than 3 times) were observed. Separation of sample was then carried out. If not specified, an average result of triplicate injections was reported.

Sample Pretreatment The tested pharmaceutical samples were all commercially available.

An aliquot of 0.8 g ground WTDT sample (passed through 50 mesh screen) was put in a conical beaker, followed by 40-min ultrasonication with exactly 10 ml of methanol as extract solvent. The extract was centrifuged. The supernatant was filtered through a 0.45 μm filter membrane and subjected to chromatographic analysis.

Two grams of GDHP sample was ground together with about 5 g diatomite powder, then heated at 90 °C for 40 min, followed by 80-min ultrasonication in a conical beaker with exactly 25 ml of methanol as extract solvent. The extract was centrifuged. The supernatant solution was filtered

* To whom correspondence should be addressed. e-mail: yubingsheng@yahoo.com

through a 0.45 μm membrane filter for GC analysis.

Results and Discussion

Optimization of GC Separation of CNMD, EL and PL Isothermal GC mode was attempted to separate the 3 objective compounds, but it was found difficult to compromise between the resolution of CNMD and EL and a rational retention factor of PL. The situation was improved by a simple temperature program, which yielded good separation of the 3 compounds (Fig. 1). The programmed GC procedure was begun at a 100 °C column temperature for 4 min and then elevated to 160 °C at a rate of 10 °C/min, where it was kept for 4 min. The injector port and FID detector temperatures were throughout kept at 220 °C and 250 °C, respectively.

Linearities and Sensitivities of the Programmed GC Method Under the chosen programmed temperature GC conditions, good linearities were observed for CNMD, EL and PL over wide ranges of concentration, respectively. The linear ranges and regression equations are given in Table 1, wherein C is the compound concentration (mg/l), and A the peak area (μV·s).

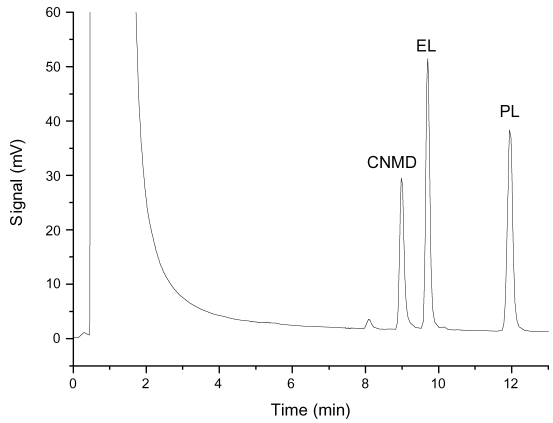

Fig. 1. Chromatogram of the Standard Mixture of CNMD, EL and PL under the Chosen GC Conditions

Detection limits (DLs), defined as signal-to-noise ratio of 3, are also given in Table 1 for the 3 objective compounds under the chosen GC conditions. The DLs were approximate to or better than that of most of the reported HPLC and GC methods.[12,13,15,18—21,23,25—27,30)]

Applications of the GC Method The present GC method was applied to separate CNMD, EL and PL in 2 herbal medicinal preparations, *i.e.*, WTDT and GDHP.

Extraction of the Objective Components: Ultrasonic extractions with different solvents (methanol, petroleum ether, and acetone) and of different treating time were tested for the 3 object components in the pharmaceutical samples. Experimental results showed that methanol yielded the best extraction for the 3 compounds in both pharmaceutical samples, and 40-min and 80-min ultrasonication with methanol as solvent were sufficient for WTDT and GDHP, respectively. Thus in this work, 40-min and 80-min ultrasonication with methanol as solvent was used to extract the objective components in WTDT and GDHP samples, respectively.

Determination of CNMD, EL and PL in WTDT and GDHP: The developed GC method was applied to determine CNMD, EL and PL in GDHP and WTDT samples. Figures 2 and 3 are typical chromatograms of WTDT and GDHP samples, respectively. The measured results are listed in Table 2.

Robustness and Recoveries of the Method Five 0.8 g WTDT or 2 g GDHP samples were ultrasonicated and separated in parallel using the developed method. The RSDs (n=5) for CNMD, EL and PL measurements were all less than 5.5%, except an elevated RSD (8.7%) for EL in the GDHP sample (Table 2).

Table 1. Linear Ranges, Regression Equations and Detection Limits (DL) of CNMD, EL and PL under the Chosen Programmed Temperature GC Conditions

Compounds	Linear ranges (mg/l)	Regression eqns.	Regression coeff. (r, n=6)	DL (mg/l)
CNMD	0.45—452	A=3785C+1386	0.9999	0.3
EL	0.31—625	A=2067C+1073	0.9999	0.2
PL	0.30—610	A=1276C+1160	0.9999	0.2

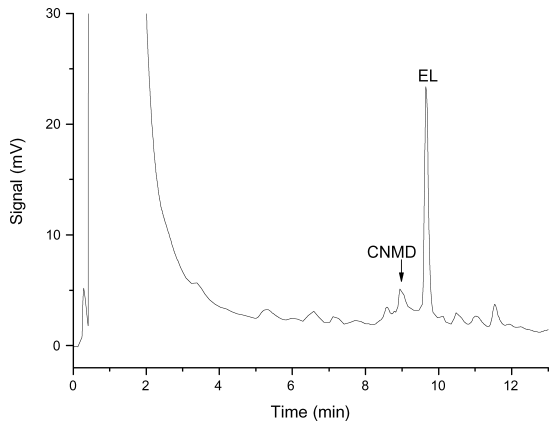

Fig. 2. Typical Chromatogram of Weitongding Tablet Sample under the Chosen GC Conditions

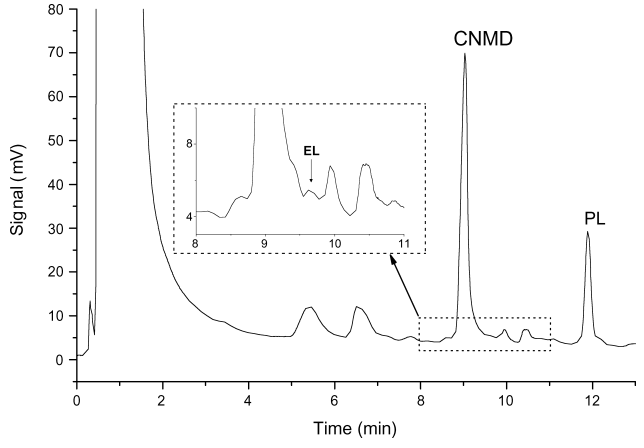

Fig. 3. Typical Chromatogram of Guifu Dihuang Pill Sample under the Chosen GC Conditions

Table 2. Measured Results of CNMD, EL and PL in GDHP and WTDT Samples

Sample	Lots No.	Compounds	Present GC method		Reference methods[1,20]	
			Mean (mg/g)	RSD% (n=5)	Mean (mg/g)	RSD% (n=3)
GDHP[a]	030803	CNMD	2.077	4.9	2.015	3.5
		EL	0.019	8.7	0.021	7.7
		PL	1.270	2.7	1.260	3.2
WTDT[b]	T23006	CNMD	0.186	5.4	0.178	5.0
		EL	1.196	4.5	1.274	3.8
		PL	ND	—	ND	—

a) GDHP: Guifu Dihuang pill, He'nan Wanxi Pharmaceutical Manufacturer, Wanxi, China. *b*) WTDT: Weitongding tablet, Guangzhou Guanghua Pharmaceutical Co., Ltd., Guangzhou, China.

Another 0.8 g WTDT or 2 g GDHP sample from the same respective source was spiked with standards of CNMD, EL and PL, respectively, and also treated and separated using the developed method. The obtained recoveries were 84—111%, 88—96% and 92—93%.

Validation of the GC Method To validate the presented GC method, the 3 objective compounds in the tested pharmaceutical samples were also individually measured using reference methods. EL was determined as the Chinese Pharmacopoeia recommended method for Dingxiang (Eugenia caryophyllata Thunb.),[1] CNMD using the Chinese Pharmacopoeia recommended method for Rouguiyou (Oleum Cinnamomi),[1] and PL using a capillary GC method as reference.[20] The results in Table 2 show good agreement between the present GC method and the reference methods.

Conclusion

The present programmed temperature capillary GC method for simultaneous determination of cinnamaldehyde, eugenol and paeonol is rather simple, sensitive and reliable. It was satisfactorily applied to simultaneously measure the 3 compounds in 2 herbal medicine preparations, *i.e.*, Guifu Dihuang pill and Weitongding tablet. The method is promising for use in the quality control of the 2 pharmaceutical preparations, especially for WTDT.

Acknowledgement This work was supported by a Foundation granted from the Educational Department of Guangdong Province, China (Project No. Q02044).

References and Notes

1) The Pharmacopoeia Committee of China, "Chinese Pharmacopoeia, Part I," Chemical Industry Publishing House, Beijing, 2000, pp. 3, 104—105, 137, 224, 546.
2) Takenaga M., Hirai A., Terano T., Tamura Y., Kitagawa H., Yoshida S., *J. Pharmacobio-Dyn.*, **10**, 201—208 (1987).
3) Shaughnessy D. T., Setzer R. W., DeMarini D. M., *Mutat. Res.*, **480**–**481**, 55—69 (2001).
4) Ohta T., Watanabe K., Moriya M., Shirasu Y., Kada T., *Mutat. Res.*, **107**, 219—227 (1983).
5) Ouattara B., Simard R. E., Holley R. A., Piette G. J., Begin A., *Int. J. Food Microbiol.*, **37**, 155—162 (1997).
6) Friedman M., Henika P. R., Levin C. E., Mandrell R. E., *J. Agric. Food Chem.*, **52**, 6042—6048 (2004).
7) Kwon J. A., Yu C. B., Park H. D., *Lett. Appl. Microbiol.*, **37**, 61—65 (2003).
8) Didry N., Dubreuil L., Pinkas M., *Pharm. Acta Helv.*, **69**, 25—28 (1994).
9) Zhang W. G., Zhang Z. S., *Yao Xue Xue Bao*, **29**, 145—148 (1994).
10) The Pharmacopoeia Committee of China, "Pharmaceutical Standards: Prescription Preparations of Chinese Medicines, Vol. 8," Beijing, 1993, p. 115.
11) Dai R., Li K., Li Q., Bi K., *J. Chromatogr. Sci.*, **42**, 207—210 (2004).
12) Luo J. P., Zhang Z. S., Tian C., Li X. M., *Zhongguo Zhong Yao Za Zhi*, **25**, 544—545 (2000).
13) Smith C. K., Cheung C., Elahi E. N., Hotchkiss S. A., *J. Chromatogr. B: Biomed. Sci. Appl.*, **758**, 249—264 (2001).
14) Okamura N., Miki H., Orii H., Masaoka Y., Yamashita S., Kobayashi H., Yagi A., *J. Pharm. Biomed. Anal.*, **19**, 603—612 (1999).
15) Wisneski H. H., Yates R. L., Davis H. M., *J. Chromatogr.*, **317**, 421—426 (1984).
16) Fischer I. U., Dengler H. J., *J. Chromatogr.*, **525**, 369—377 (1990).
17) Wu X., Chen H., Chen X., Hu Z., *Biomed. Chromatogr.*, **17**, 504—508 (2003).
18) Zhao X., Sun Y., *Anal. Sci.*, **19**, 1313—1315 (2003).
19) Riley C. M., Ren T. C., *J. Chromatogr.*, **489**, 432—437 (1989).
20) Miao H. J., Liu Z. L., Li Y. H., *Yao Xue Xue Bao*, **32**, 928—930 (1997).
21) Wang S. Y., Chen P. F., Chang S. T., *Bioresour. Technol.*, **96**, 813—818 (2005).
22) Usta J., Kreydiyyeh S., Bajakian K., Nakkash-Chmaisse H., *Food Chem. Toxicol.*, **40**, 935—940 (2002).
23) Friedman M., Kozukue N., Harden L. A., *J. Agric. Food Chem.*, **48**, 5702—5709 (2000).
24) Rastogi S. C., Heydorn S., Johansen J. D., Basketter D. A., *Contact Dermatitis*, **45**, 221—225 (2001).
25) Rastogi S. C., Johansen J. D., Frosch P., Menne T., Bruze M., Lepoittevin J. P., Dreier B., Andersen K. E., White I. R., *Contact Dermatitis*, **38**, 29—35 (1998).
26) Lee S. J., Noble A. C., *J. Agric. Food Chem.*, **51**, 8036—8044 (2003).
27) Aznar M., Lopez R., Cacho J. F., Ferreira V., *J. Agric. Food Chem.*, **49**, 2924—2929 (2001).
28) Akeng'a T. A., Chhabra S. C., *Afr. J. Med. Med. Sci.*, **26**, 79—81 (1997).
29) Stanfill S. B., Ashley D. L., *J. Agric. Food Chem.*, **48**, 1298—1306 (2000).
30) Myint S., Daud W. R., Mohamad A. B., Kadhum A. A., *J. Chromatogr. B: Biomed. Appl.*, **679**, 193—195 (1996).
31) Mitic N., Pavlovic V., Djordjevic M., *Rev. Odontostomatol.* (Paris), **12**, 452—460 (1983).
32) Zhou H. B., *Academic J. Guangdong College of Pharmacy*, **16**, 218—221 (2000).