

Use of caffeine as a probe for rapid determination of cytochrome P-450 CYP1A2 activity in humans¹

OU-YANG Dong-Sheng, HUANG Song-Lin, XIE Hong-Guang, WANG Chuan-Yue, ZHOU Hong-Hao²
(Pharmacogenetics Research Institute, Hu-nan Medical University, Changsha 410078, China)

KEY WORDS caffeine; xanthines; cytochrome P-450 CYP1A2; high pressure liquid chromatography; pharmacokinetics

AIM: To develop a rapid HPLC method for the determination of cytochrome P-450 CYP1A2 activity. **METHODS:** A 300- μ L plasma was prepared by extraction with 5-mL chloroform/isopropanol (9:1), and β -hydroxyethiotheophylline was added as internal standard (IS). Samples were separated on an ODS column by a gradient elution system, of which mobile phase consisted of 0.05 % acetic acid, acetonitrile, and methanol. The compounds of interest were monitored at 282 nm by UV detector. **RESULTS:** No potential interfering peaks were found. Paraxanthine (17X), IS and caffeine (137X) were rapidly eluted with baseline resolution, and their retention time was less than 13 min. The detection limits of both 17X and 137X were 0.1 μ mol \cdot L⁻¹. Linear relations ranged over 1 - 100 μ mol \cdot L⁻¹ and 1 - 200 μ mol \cdot L⁻¹ with correlation coefficient of 0.9999 and 0.9987, respectively, for 17X and 137X. The coefficients of variation were within 6 % for 17X, and 10 % for 137X. The average recoveries for both compounds were ranged from 96 % to 108 %. **CONCLUSION:** This method is sensitive and rapid, and can be used for population studies of CYP1A2.

Cytochrome P-450 CYP1A2 is a liver microsomal enzyme involved in the metabolic activation of environmental carcinogens, including heterocyclic amines, arylamine, and nitrosamine, and in the biotransformation of drugs^[1]. Up to 70-fold variation exists in the activities of this enzyme in different individuals^[2]. A test for the catalytic activity of

CYP1A2 is desirable to estimate the individual risk of certain environment-induced toxicity or cancers and to reduce adverse reactions of affected drugs. Caffeine (137X) is most commonly used as an *in vivo* probe of CYP1A2 because of its low toxicity and better compliance^[3]. Several metabolic indices of caffeine have been proposed to reflect CYP1A2 activity^[3]. Of them, paraxanthine/caffeine ratio (17X/137X) in the postdose plasma or saliva is currently recognized as the best buy^[4-7]. Several HPLC methods were available for determining 137X and its metabolites but with low sensitivity, time-consuming, or poor resolution^[2,6-8], a rapid and sensitive HPLC method is required, especially during population survey and routine determination of CYP1A2 activity. This paper described a rapid and sensitive RP-HPLC method for simultaneous quantitation of 17X and 137X to measure the activity of CYP1A2 in humans.

MATERIALS AND METHODS

Chemicals and drugs Standard products 17X, 137X, and β -hydroxy-ethyltheophylline (HT) were purchased from Sigma Chemical Co. Caffeine (300 mg) capsules were provided kindly by Hunan Pharmaceutical Co (Changsha, China). Methanol and acetonitrile were of HPLC grade, and other reagents were of AR grade unless indicated. Water was purified by osmosis and redistillation.

Standard solutions Stock solutions of 17X, 137X, and HT (1 mmol \cdot L⁻¹) were prepared in water and kept at -20 °C. Working solutions of 17X and 137X were freshly prepared in blank plasma, except for HT in water.

Sample preparation Plasma 300 μ L was mixed with HT (an internal standard, IS, 100 μ mol \cdot L⁻¹) 100 μ L, and ammonium sulfate 300 mg. The sample was extracted with 5 mL chloroform/isopropanol (9:1, vol/vol) and vortexed for 1 min. After centrifugation at 1500 \times g for 15 min, the organic phase was evaporated to dryness at 45 - 50 °C under N₂. The residue was dissolved in a 100 μ L eluent and 20 μ L aliquot was used for HPLC.

HPLC It consisted of HP 1050 Series, a reversed-phase column (Spherisorb ODS-2, 250 mm \times 4 mm ID, 5 μ m particle size) and Chemstation controller. The eluent was composed of

¹ Project supported by the National Natural Science Foundation of China, No C39200154 and F99340230, and by China Medical Board of New York, USA Medical Research 92-5691.

² Correspondence to Prof ZHOU Hong-Hao. P.hn 86-731-444-7233. Fax 86-731-447-1339. E-mail hhzhou@public.cs.hn.cn

Received 1997-10-06

Accepted 1997-06-17

0.05 % acetic acid, acetonitrile, and methanol at the ratio of 81.5:8.5:10 for 0–5 min, then 72:18:10 afterwards. The flow rate was $0.7 \text{ mL} \cdot \text{min}^{-1}$, and UV detection was set at 282 nm.

Validation study A series of spiked standards ($1.0 - 200.0 \mu\text{mol} \cdot \text{L}^{-1}$) of 17X and 137X were prepared with blank heparinized plasma from volunteers refrained from the caffeine-containing foodstuffs and drinks for at least 5 d. Each compound was quantified by the peak area ratio using a calibration curve. Three standard solutions containing, respectively, 5.0, 25.0, and $100.0 \mu\text{mol} \cdot \text{L}^{-1}$ of both 17X and 137X were prepared to test the accuracy and precision of the assay. The lower limit of detection was defined as a signal-to-noise ratio of 4:1. The potential interference from endogenous sources was examined with blank plasma.

Applications of the method Plasma was collected from the volunteers 5–7 h after *po* a 300-mg capsule of caffeine, and stored at -20°C .

RESULTS AND DISCUSSION

HPLC According to the representative chromatograms obtained from the blank plasma of volunteers (Fig 1A), the spiked plasma with 17X, HT, and 137X (Fig 1B), and an unknown plasma during 5–7 h after oral administration of 300 mg caffeine (Fig 1C), no endogenous interfering peaks were found in the blank sample. The 17X, IS, and 137X were rapidly eluted with a complete resolution and sharp symmetrical peaks. The retention time for 17X, IS, and 137X was within 13 min, which was much shorter than that reported by others^(2,7).

Calibration curve The calibration curves for 17X and 137X were linear over the range of $1.0 - 100.0 \mu\text{mol} \cdot \text{L}^{-1}$ and $1.0 - 200.0 \mu\text{mol} \cdot \text{L}^{-1}$ with correlation coefficient (r) of 0.9999 and 0.9987, respectively (Fig 2).

Validation test The average recoveries of 17X and 137X were ranged from 96 % to 108 % (Tab 1). Coefficients of both intra- and inter-day variations (CV) were $<6\%$ for 17X, and $<10\%$ for 137X (Tab 1). The detection limits of 17X and 137X were up to $0.1 \mu\text{mol} \cdot \text{L}^{-1}$, and were lower than the previous data⁽⁶⁾.

Application of the method This method has been applied to a population study (unpublished). The detected concentration of the subjects was from 3.5 to $21.5 \mu\text{mol} \cdot \text{L}^{-1}$ for 17X, and from 7.1 to $81.3 \mu\text{mol} \cdot \text{L}^{-1}$ for 137X, respectively. The results

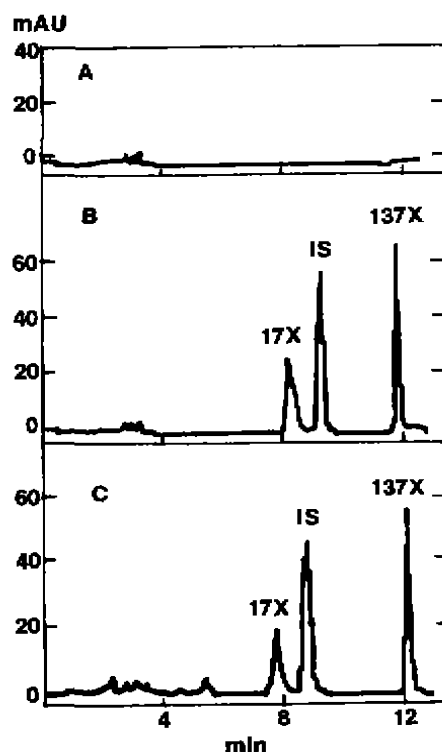


Fig 1. Chromatograms of blank plasma (A), spiked plasma (B), and unknown plasma from a subject who took *po* caffeine 300 mg (C).

Tab 1. Coefficients of variation (CV, %), and recovery ($\bar{x} \pm s$, %) in determination of 17X and 137X in spiked plasma.

	Added/ $\mu\text{mol} \cdot \text{L}^{-1}$		
	5	25	100
Recovery/% ($n=10$)			
17X	96.0 ± 4.6	101.6 ± 1.9	99.8 ± 2.2
137X	108.0 ± 7.9	98.8 ± 6.0	98.8 ± 2.0
Intra-day CV ($n=5$)			
17X	3.9	1.7	3.3
137X	7.4	5.2	1.9
Inter-day CV ($n=5$)			
17X	5.6	2.2	0.1
137X	9.3	6.4	0.4

indicated that CYP1A2 activity appeared to show genetic polymorphism, with the incidence of PM (poor metabolizers) being about 5 %. In summary, we developed a rapid and sensitive method that might be suited for population studies.

ACKNOWLEDGMENTS Prof DENG Han-Wu and LI Yuan-Jian of Department of Pharmacology for providing sampling field, Mrs WANG Dan and JIANG

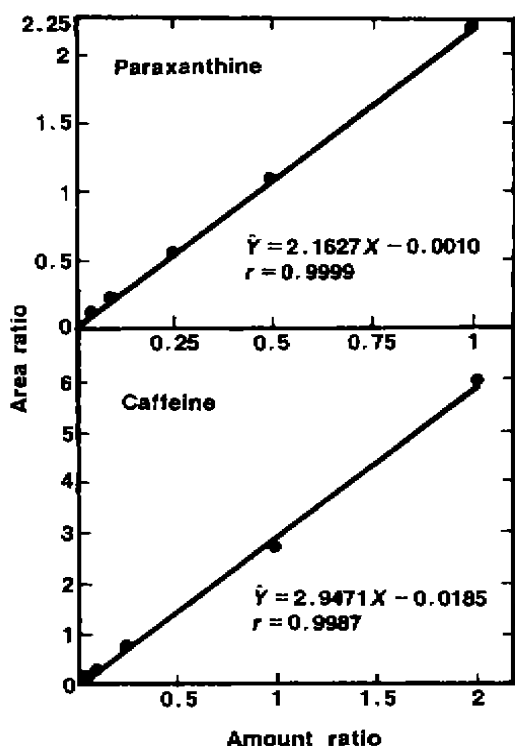


Fig 2. Calibration curves for paraxanthine and caffeine.

Chang-Hong for preparing with experimental materials, Mr QING Xu-Ping, SUN Jie and Miss WU Zhong-Luan and LU Rong for collecting samples, Miss ZHU Bing for typing, and all the volunteers for kind cooperation.

REFERENCES

- 1 Eaton DL, Gallagher EP, Bammler TK, Kunze KL. Role of cytochrome P450A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity. *Pharmacogenetics* 1996; 6: 259-74.
- 2 Butler MA, Lang NP, Young JF, Caporaso NE, Vaneis P, Hayes RB, et al. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics* 1992; 2: 116-27.
- 3 Ou-Yang DS, Xie HG, Zhou HH. Use of caffeine as a probe for the determination of enzyme CYP1A2 activity in humans. *Hunan Med J* 1997; 14: 40-1.

- 4 Fuhr U, Rost KL. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics* 1994; 4: 109-16.
- 5 Rostami-Hodjegan A, Nurminen S, Jackson PR, Tucker GT. Caffeine urinary metabolite ratios as markers of enzyme activity: a theoretical assessment. *Pharmacogenetics* 1996; 6: 121-49.
- 6 Dobrocky P, Bennett PN, Notarianni LJ. Rapid method for the routine determination of caffeine and its metabolites by high-performance liquid chromatography. *J Chromatogr Biomed Appl* 1994; 652: 104-8.
- 7 Roberts EA, Furuya KN, Tang BK, Kalow W. Caffeine biotransformation in human hepatocyte lines derived from normal liver tissue. *Biochem Biophys Res Commun* 1994; 201: 559-66.
- 8 Fuhr U, Wolff T, Harder S, Schymanski P, Staib AH. Quinolone inhibition of cytochrome P-450-dependent caffeine metabolism in human liver microsomes. *Drug Metab Dispos* 1990; 18: 1005-10.

ψψ-ψ6

用咖啡因作探药, 快速测定人体细胞色素 P-450 CYP1A2 酶的活性¹

R392-33

欧阳冬生, 黄松林, 谢红光, 王传跃, 周宏灏²
(湖南医科大学遗传药理研究所, 长沙 410078, 中国)

关键词 咖啡因; 黄嘌呤类; 细胞色素 P-450 CYP1A2; 高压液相色谱法; 药物动力学

目的: 建立快速测定细胞色素 P-450 CYP1A2 酶活性的高压液相色谱方法. 方法: 取 300 μL 血浆样品, 用 β-羟乙基茶碱作内标, 经 5 mL 氯仿/异丙醇(9:1)萃取处理后, 用 0.05 % 的乙酸、乙腈和甲醇作为基本流动相, 采用梯度洗脱程序在 ODS 柱上分离待测组分, 紫外检测波长 282 nm. 结果: 无内源性物质干扰测定. 次黄嘌呤、内标和咖啡因快速基线分离, 三者的保留时间均小于 13 分钟. 次黄嘌呤和咖啡因的检测下限均为 0.1 μmol·L⁻¹, 线性范围分别为 1-100 μmol·L⁻¹ 和 1-200 μmol·L⁻¹, 相关系数分别为 0.9999 和 0.9987, 变异系数分别小于 6 % 和 10 %. 两者的平均相对回收率为 96 % - 108 %. 结论: 本方法快速、灵敏, 可用于人群 CYP1A2 酶活性研究.