ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1): 87-91
http://www.chinaphar.com

Restricted-access media high pressure liquid chromatography *vs* fluorescence polarization immunoassay for analysis of carbamazepine in human plasma[1]

MA Jun, ZHU Peng-Ling[2] (*Department of Chemistry, Lanzhou University, Lanzhou 730000, China*);
XIE Jing-Wen, JIA Zheng-Ping, YANG Jin-Sheng
(*Department of Pharmacy, General Hospital of Lanzhou Command of PLA, Lanzhou 730050, China*)

KEY WORDS high pressure liquid chromatography; restricted-access media; fluorescence polarization immunoassay; carbamazepine

ABSTRACT

AIM: To compare restricted-access media high performance liquid chromatographic (RAM-HPLC) method with fluorescence polarization immunoassay (FPIA) for analysis of carbamazepine (CBZ) in human blood. **METHODS**: An RAM-HPLC method was established for the determination of CBZ in plasma. **RESULTS**: The two methods do not need sample clean-up prior to analysis and they have almost 100 % recovery and good reproducibility. There is a good correlation between the CBZ concentration in venous plasma samples determined by FPIA and that in both venous and fingertip plasma samples obtained by RAM-HPLC, the correlation coefficients being 0.989 and 0.995, respectively. It is shown by *t*-test that the data sets of venous and fingertip plasma samples given by RAM-HPLC are consistent with each other but significantly different from the results obtained by FPIA. **CONCLUSION**: Both direct injection RAM-HPLC and FPIA may be applied in determining CBZ in therapeutic drug monitoring (TDM). FPIA is well-suited to the routine TDM. RAM-HPLC is more useful in TDM related research and especial cases.

INTRODUCTION

Therapeutic drug monitoring (TDM) is necessary to treatment when drugs have narrow therapeutic ranges. Carbamazepine (CBZ) is a drug most used for the treatment of epilepsy and its dosage has been optimized by monitoring its plasma concentrations for about the past two decades[1,2]. Some high performance liquid chromatographic (HPLC) methods have been developed with the increasing demand for the determination of CBZ in biological fluids[3-5]. In the case of reversed phase liquid chromatography, however, the proteins in the samples can be absorbed on the surface of stationary phase, resulting in the decrease of efficiency and column clogging. Thus, a pretreatment of sample prior to chromatographic analysis is required to remove the proteins in biological fluids.

The different types of restricted-access media (RAM) used for direct injection analysis of biological fluids have been proposed since 1980s, including protein-coated octadecylsilane, internal surface reversed phase, shielded hydrophobic phase, mixed-functional phase, dual-zone packing material, semipermeable-surface reversed phase and so on[6,7]. The phases have the pore size small enough to restrict the access of macromolecules while small molecules can penetrate the particulate and interact with the bonded phases to be retained. The destructive accumulation of proteins is avoided or reduced owing to the existence of hydrophilic external surface. The various RAM were used for the determination of CBZ in biological fluids[8,9].

Meanwhile, the fully automated instrument based on the fluorescence polarization immunoassay (FPIA) principles has been widely used for the therapeutic drug monitoring (TDM) of CBZ. The instrument is easy to be operated and no sample preparation is required. On the other hand, FPIA is relatively expensive to run and the technique is not specific. Antibodies may cross-react with metabolites and drugs structurally close to parent drugs, or endogenous substances, giving high results[1].

In this work, the direct injection analysis of human

[1] Project supported by the Ninth Five-year Medicine Research Fundation in the Force, № 98M018.
[2] Correspondence to Prof ZHU Peng-Ling. Phn 86-931-266-2432. Fax 86-931-891-1286. E-mail MB1642@public.lz.gs.cn
Received 2001-02-26 Accepted 2001-09-14

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

peripheral blood was carried out on the shielded hydrophobic phase of RAM. The detailed description about the stationary phase is given in Ref 10. The purposes of this work are: to compare RAM-HPLC method with FPIA for the clinical assay of CBZ and to examine whether there is difference between venous and fingertip plasma samples for the determination of CBZ.

MATERIALS AND METHODS

Reagents Diazepam as internal standard (IS) was provided by National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile (ACN) was obtained from Siyou Company (Tianjin, China). All other reagents were of analytical grade from a variety of suppliers.

Internal standard solution of diazepam 15 mg/L: 1.5 mg of diazepam was dissolved into 15 mL of methanol in a 100 mL volumetric flask and water was added to the mark.

Equipment A component HPLC system consisted of LC-6A pump (Shimadzu, Kyoto, Japan), Rheodyne 7125 injection valve with 20 μL loop (Catati, CA, USA), and Model SPD-6A UV/visible spectrophotometer detector (Shimadzu, Kyoto, Japan) operated at 280 nm. A C-R3A chromatopac recorder/integrator (Shimadzu, Kyoto, Japan) was connected to the output of the detector. The chromatographic conditions were set up as follows: analytical column, Hisep shielded hydrophobic phase[10], 15 cm × 0.46 cm, 5 μm; guard column, Hisep shielded hydrophobic phase, 2 cm × 0.46 cm (Supelco, Bellefonte, PA, USA), mobile phase, ACN/ammonium acetate 0.1 mol/L, 12/88 (v/v); flow rate, 1.5 mL/min; temperature, ambient; injection volume, 20 μL.

FPIA was performed on TDX machine (Abbott Laboratories Irving, TX, USA). CBZ reagent pack (Abbott Laboratories, North Chicago, IL, USA) includes CBZ calibrators (0, 2, 4, 8, 12, and 20 mg/L solutions of CBZ in serum) and controls (3, 6, and 16 mg/L solutions of CBZ in serum).

Procedures IS solution of diazepam 20 μL was added into 20 μL of CBZ calibrators and controls each, then the mixtures was vortexed. Under the specified chromatographic conditions, each CBZ calibrator samples were injected twice, and the average of CBZ and IS peak area ratio was regressed against the concentration of CBZ to give the equation of calibration curve. The interday and intraday variations were calculated according to the results obtained by three injections of CBZ controls.

The instructions for the determination of CBZ described in the TDX Assay Manual were followed scrupulously to carry out FPIA.

Epileptic patients took CBZ but did not take diazepam in the hospital. Venous blood 2 mL and 40 μL of peripheral blood drawn from fingertip were collected simultaneously from each epileptic patient after CBZ concentration achieved a stable state. The blood samples were centrifuged at 10 000 × *g* for 5 min. The centrifuged venous plasma was divided into two parts used for the determination by FPIA and RAM-HPLC. The centrifuged peripheral plasma samples were analyzed by RAM-HPLC only.

RESULTS

Under the specified chromatographic conditions, CBZ and diazepam were eluted at 10.5 min and 15.5 min, respectively, the chromatogram shown in Fig 1. The proteins in the sample passed through the column without retention. Over 2 – 20 mg/L of CBZ concentration in human serum, the equation of calibration curve is $Y = 0.151X - 0.0849$ with correlation coefficient 0.998, where X and Y are CBZ concentration and peak area ratio of CBZ and diazepam, respectively. The detection limit

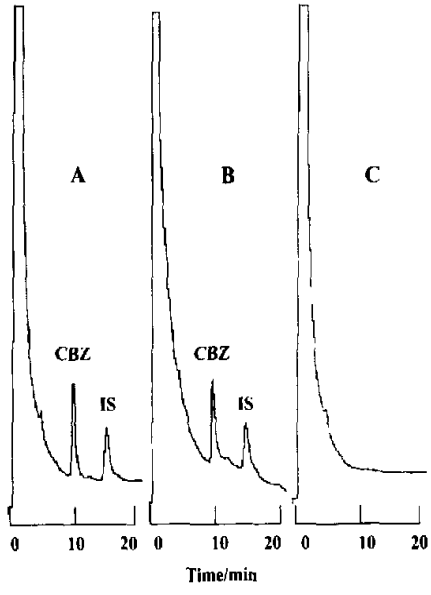

Fig 1. Chromatograms of direct injection analysis of CBZ calibrator (8 mg/L) containing internal standard (15 mg/L) (A), plasma from a patient after oral administration of CBZ (B), and blank serum (C).

of CBZ was evaluated as 0.5 mg/L.

The accuracy and precision of RAM-HPLC and FPIA methods were calculated according to the three injections of CBZ controls, shown in Tab 1 and Tab 2.

The CBZ concentrations in the plasma samples of the clinic patients were determined by means of RAM-HPLC (venous and fingertip bloods) and FPIA (venous blood). The correlation between the determined results is shown in Fig 2 and their statistics are summarized in Tab 3. It is indicated by t-test ($\alpha = 0.05$) that the CBZ concentrations determined by RAM-HPLC are statistically different from those given by FPIA, but there is no significant difference between the results of venous and fingertip blood samples obtained by RAM-HPLC.

DISCUSSION

Both RAM-HPLC and FPIA do not need sample clean-up prior to analysis and their analysis time is roughly the same. Compared with FPIA, RAM-HPLC has the following advantages: less sample size, high specificity and ability to assay several compounds simultaneously (see Fig 1). In contrast to traditional HPLC, RAM-HPLC is simple and timesaving, only needs small sample size and has almost 100 % recovery due to eliminate the need for biological sample preparation. Advantages of FPIA are quick, easy to perform, and permitting much higher sample throughput.

It can be seen from Tab 3 that the FPIA assay gives higher CBZ concentration than that determined by RAM-HPLC, based on both venous and fingertip blood samples of the same epileptic patients. The overestimation of CBZ content is probably caused by one or more CBZ metabolites[4,5,11]. Because of its specificity, HPLC can be used as reference method to develop a new immunoassay.

The consistency of CBZ concentration in the venous blood with that in the fingertip blood means that TDM of CBZ can be carried out by RAM-HPLC, based on small volume of peripheral blood. That is particularly significant for infants, children, and old patients, from whom it is sometimes difficult to collect blood samples at an appropriate volume.

With regard to analysis of CBZ and another drugs in TDM, RAM-HPLC and FPIA have their particular advantages. They should be regarded as complementary to each other. FPIA is well-suited to the routine monitoring of drugs in a clinical setting. RAM-HPLC are more useful in TDM related research and especial cases.

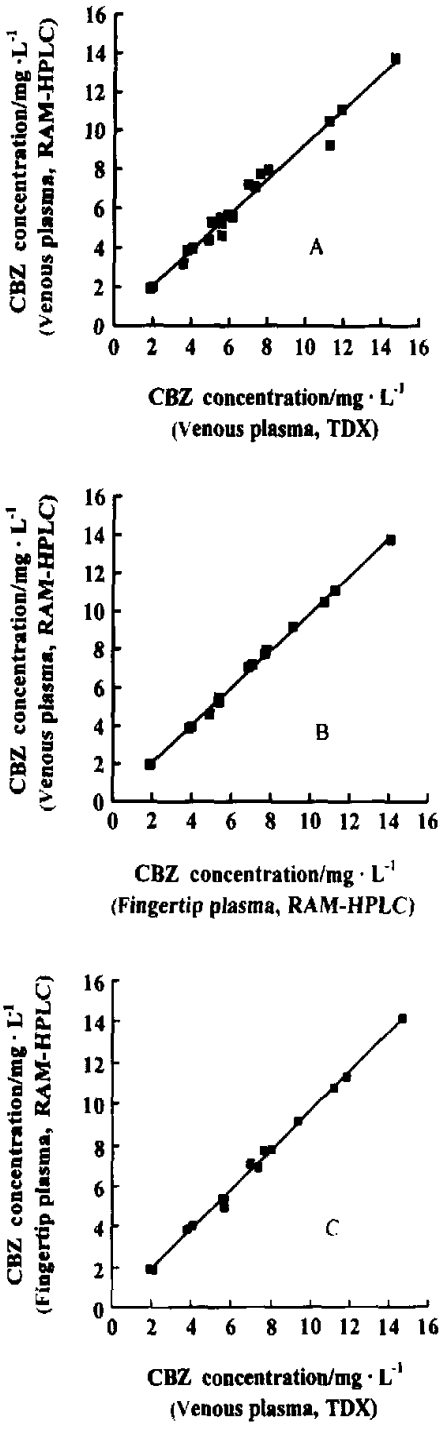

Fig 2. Correlation of CBZ concentrations in patient plasma measured by RAM-HPLC and TDX.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Tab 1. Precision of RAM-HPLC method.

Interday

Expected concentration of CBZ controls/mg·L^{-1}	Measured concentration of CBZ controls[1]/mg·L^{-1} d 1	d 2	d 3	$\bar{x} \pm s$	RSD/%
3.00	3.12 (4.0)[2]	2.83 (5.7)	3.07 (2.3)	3.01 ± 0.13	4.2
6.00	6.14 (2.3)	6.19 (3.2)	5.77 (3.8)	6.03 ± 0.19	3.1
16.00	16.18 (1.1)	16.57 (3.6)	15.94 (0.4)	16.23 ± 0.26	1.6

Intraday

Expected concentration of CBZ controls/mg·L^{-1}	Measured concentration of CBZ controls[1]/mg·L^{-1} Test 1	Test 2	Test 3	$\bar{x} \pm s$	RSD/%
3.00	3.07 (2.3)	3.15 (5.0)	3.11 (3.7)	3.11 ± 0.03	1.1
6.00	5.77 (3.8)	6.15 (2.5)	5.81 (3.2)	5.91 ± 0.17	2.8
16.00	15.94 (0.4)	15.75 (1.6)	16.51 (3.2)	16.07 ± 0.32	2.0

1) The concentration was obtained by means of the linear regression of CBZ calibrators.
2) The value in the bracket is % accuracy calculated according to [(measured value − expected value)/expected value] × 100 %.

Tab 2. Precision of FPIA technique.

Interday

Expected concentration of CBZ controls/mg·L^{-1}	Measured concentration of CBZ controls[1]/mg·L^{-1} d 1	d 2	d 3	$\bar{x} \pm s$	RSD/%
3.00	2.98 (0.7)[1]	3.02 (0.7)	3.01 (0.3)	3.00 ± 0.02	0.6
6.00	6.04 (0.7)	6.03 (0.5)	5.94 (1.0)	6.00 ± 0.04	0.7
16.00	15.81 (1.2)	16.22 (1.4)	15.91 (0.6)	15.98 ± 0.17	1.1

Intraday

Expected concentration of CBZ controls/mg·L^{-1}	Measured concentration of CBZ controls[1]/mg·L^{-1} Test 1	Test 2	Test 3	$\bar{x} \pm s$	RSD/%
3.00	3.03 (1.0)	3.00 (0.0)	2.98 (0.7)	3.00 ± 0.02	0.7
6.00	6.09 (1.5)	6.17 (2.8)	6.04 (0.7)	6.10 ± 0.05	0.9
16.00	16.33 (2.1)	16.29 (1.8)	15.81 (1.2)	16.14 ± 0.24	1.5

1) The value in the bracket is % accuracy calculated according to [(measured value − expected value)/expected value] × 100 %.

Tab 3. Comparison between the measured results obtained by RAM-HPLC and FPIA and for venous and fingertip blood.

Comparison	Number of samples	Regression analysis Slope b	Intercept a	Correlation coefficient γ	*t*-test *P*
RAM-HPLC for venous (*Y*) *vs* FPIA for venous (*X*)	20	0.908	0.195	0.989	< 0.05
RAM-HPLC for fingertip (*Y*) *vs* FPIA for venous (*X*)	15	0.935	0.125	0.995	< 0.05
RAM-HPLC for venous (*Y*) *vs* RAM-HPLC for fingertip (*X*)	15	0.968	0.132	0.998	> 0.05

REFERENCES

1 Mehta AC. The how and why of therapeutic drug monitoring. Anal Proc 1995; 32: 347 - 51.
2 Rambeck B, May TW, Jürgens U, Blankenhorn V, Jürges U, Korn-Merker E, *et al*. Comparison of phenytoin and carbamazepine serum concentrations measured by high-performance liquid chromatography, the standard TDx assay, the enzyme multiplied immunoassay technique, and a new patient-side immunoassay cartridge system. Ther Drug Monit 1994; 16: 608 - 12.
3 Reith DM, Cannell GR. An HPLC assay for carbamazepine phase I metabolites and their glucuronides in urine. J Liq Chromatogr Relat Technol 1999; 22: 1907 - 18.
4 Tsaprounis CK, Kajbaf M, Corrod JW. Simultaneous determination of carbamazepine and its major metabolites in human plasma and urine by HPLC. J Clin Pharm Ther 1991; 16: 257 - 62.
5 Bonato PS, Lanchote VL, de Carvalho D, Ache P. Measurement of carbamazepine and its main biotransformation products in plasma by HPLC. J Anal Toxicol 1992; 16: 88 - 92.
6 Zhu PL, Wang DJ, Qu Y. Stationary phases for use in direct injection analysis of biological fluids. Chin Pharm J 1994; 29: 129 - 33.
7 Boos KS, Rudolpbi A. The use of restricted access media in HPLC, part Ⅰ-classification and review. LC-GC 1997; 15: 602 - 11.
8 Rudolpbi A, Boos KS. The use of restricted access media in HPLC, part Ⅱ-applications. LC-GC 1997; 15: 814 - 23.
9 Ma J, Xie JW, Sun WS, Jia ZP. Synthesis and evaluation of restricted access stationary phases. Acta Pharm Sin 1998; 33: 369 - 72.
10 Gisch D, Hunter BT, Feibush B. Shielded hydrophobic phase: a new concept for direct injection analysis of biological fluids by high-performance liquid chromatography. J Chromatogr 1988; 433: 264 - 8.
11 Oeltgen DJ, Shank JWA, Blouin RA, Clark T. Clinical evaluation of the Abbott TDx fluorescence polarization immunoassay analyzer. Ther Drug Monit 1984; 6: 360 - 7.

浸透限制固定相高压液相色谱法和荧光偏振免疫法测定人血中卡马西平浓度的比较[1]

R96 A

马 骏，朱彭龄[2]（兰州大学化学系，兰州 730000，中国）；谢景文，贾正平，杨金升（兰州军区总医院药剂科，兰州 730050，中国）

关键词 高压液相色谱法；浸透限制固定相；荧光偏振免疫测定；卡马西平

目的：比较浸透限制固定相高效液相色谱法（RAM-HPLC）与荧光偏振免疫法（FPIA）测定人血中的卡马西平（CBZ）浓度．**方法**：建立一种直接进样 RAM-HPLC 法测定病人静脉和指端血浆的 CBZ 浓度．**结果**：两种测定方法都不需样品前处理，有好的重现性和近 100 % 的回收率．FPIA 测定静脉血样 CBZ 的结果与 RAM-HPLC 分别测定静脉和指端血样 CBZ 的结果有良好的相关性（$R = 0.989$, 0.995），但相差显著（$P < 0.05$）；而 RAM-HPLC 法测定静脉和指端血样两组数据间相差不显著（$P > 0.05$）．**结论**：RAM-HPLC 和 FPIA 法均可测定 CBZ 浓度．在治疗药物监测中，FPIA 更适合常规的监测；RAM-HPLC 法更适用于相关研究和特殊病例监测，这里我们成功地应用于测定人外周微量血样中 CBZ 的浓度．

（责任编辑 吕 静）