Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1088 (2005) 146–151
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Measurement and pharmacokinetics of vincamine in rat blood and brain using microdialysis

Ying-Ping Juan [a], Tung-Hu Tsai [a, b, *]

[a] *Institute of Traditional Medicine, National Yang-Ming University, Taipei 112, Taiwan*
[b] *National Research Institute of Chinese Medicine, 155-1 Li-Nong Street Section 2, Taipei 112, Taiwan*

Available online 5 February 2005

Abstract

Vincamine is an alkaloid compound derived from the *Vinca minor* plant. Since little is known concerning its pharmacokinetics and appropriate analytical method, this study focuses on its pharmacokinetics as well the possible roles of the multidrug transporter P-glycoprotein on its distribution and disposition. We develop a rapid and sensitive method using a microdialysis coupled with liquid chromatography for the concurrent determination of unbound vincamine in rat blood and brain. Microdialysis probes were simultaneously inserted into the jugular vein toward heart and brain hippocampus of male Sprague–Dawley rats for sampling in biological fluids following the administration of vincamine (10 and 30 mg/kg) through the femoral vein. Samples were eluted with a mobile phase containing methanol–1% diethylamine (pH 7.15) in water (75:25, v/v) and the flow rate of the mobile phase was 0.7 ml/min. Pharmacokinetic parameters of vincamine were derived using compartmental model. The decline of protein-unbound vincamine in the hippocampus and blood suggested that there was rapid exchange and equilibration between the peripheral compartment and the central nervous system. In the presence of cyclosporine, unbound vincamine levels in both blood and brain were significantly increased.

Keywords: Blood–brain barrier; Cyclosporine; Microdialysis; P-glycoprotein; Pharmacokinetics; Vincamine

1. Introduction

Vincamine (Fig. 1; 14,15-dihydro-14hydroxyeburnamenine-14-carboxylic acid methyl ester) is an alkaloid compound derived from the *Vinca minor* plant. which has been used for the prevention or the therapy for cerebrovascular insufficiencies and disorders. The elimination half-life is approximately 2 h after vincamine oral administration. Vincamine is mainly metabolized in the liver and excreted by the kidneys and it may also go through cerebral metabolism [1]. Following intraperitoneal injection, vincamine or its metabolites have been verified with the autoradiographic method to penetrate the blood–brain barrier (BBB) [2].

Methods for the determination of vincamine in blood or plasma have been reported using thin-layer chromatography [3], mass spectrometry [4], voltammetric detection [5], gas chromatography [6,7], liquid chromatography [5,8,9] and separation of its enantiomers [10].

In the pharmacokinetic profile of vincamine, the compound is about 64% bound to plasma proteins, and 6% bound to erythrocytes [11]. To date, no assay method has been reported for the sampling and measurement of protein-unbound vincamine in biological fluids. To determine the pharmacokinetics of the protein-unbound form of an analyte in the body, we employed in vivo microdialysis techniques to obtain the protein-free analyte from simultaneously derived rat blood and brain samples [12,13]. Microdialysis sampling techniques were originally developed to allow in vivo sampling of neurotransmitters released in the brain [14,15], and techniques have subsequently been extended to encompass pharmacological and pharmacokinetic studies [12,13].

The aim of this study is to investigate the disposition of unbound vincamine and its distribution into the brain. A rapid and sensitive liquid chromatographic system coupled to microdialysis is demonstrated for the determination of protein-unbound vincamine in rat blood and brain. In

* Corresponding author. Tel.: +886 2 2820 1999x8091; fax: +886 2 2826 4276.
E-mail address: thtsai@ym.edu.tw (T.-H. Tsai).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.043

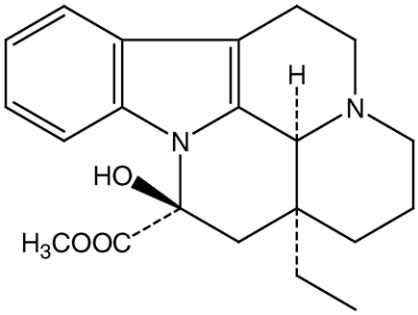

Fig. 1. Chemical structure of vincamine.

addition, further exploration of the mechanism for BBB penetration is also observed by comparing the pharmacokinetics of vincamine both with and without cyclosporine, a P-glycoprotein inhibitor.

2. Experimental

2.1. Chemicals and reagents

Vincamine and cyclosporine (sandimmun) were purchased from Sigma (St. Louis, MO, USA) and Novartis Pharma (Basle, Switzerland), respectively. Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Animals

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. Animals had free access to food (Laboratory rodent diet No. #5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to experimentation, at which time only food was removed. The rats were initially anaesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.), and remained anaesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. During the experiment, the rat body temperature was maintained at 37 °C using a heating pad.

2.3. Chromatography

The chromatographic system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an on-line injector (CMA/160, Stockholm, Sweden) equipped with a 20 μl sample loop and an ultraviolet detector (Varian, Walnut Creek, CA, USA) for biological sample analysis. A Waters photodiode array detection (Waters 2695 module coupled to 2696 detector; Waters Millipore, Milford, MA, USA) was applied to detect the maxima absorption wavelength of vincamine. Separation and quantification of vincamine in the blood and brain dialysates were achieved using a reversed phase Merck LiChroCart C_{18} endcapped column (250 mm × 4.6 mm I.D.; particle size 5 μm, Darmstadt, Germany) maintained at ambient temperature. Dialysis samples were eluted with a mobile phase containing 1% diethylamine in water (pH 7.15)–methanol (25:75, v/v), and the flow rate of the mobile phase was 0.7 ml/min. The UV detector wavelength was set at 220 nm. This mobile phase was filtered with a 0.45 μm Millipore membrane prior to being used for elution. The output signal from the HPLC-UV detector was recorded using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Microdialysis experiment

Blood and brain microdialysis systems consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and the appropriate microdialysis probes [12]. The dialysis probes for blood (10 mm in length) and brain (3 mm in length) were made of silica glass capillary tubing arranged in a concentric design [16,17]. Their tips were covered by dialysis membrane (150 μm outer diameter with a nominal molecular weight cut-off of 13 000, Spectrum Co, Laguna Hills, CA, USA) and all unions were cemented with epoxy. To allow adequate time for the epoxy to dry, the probes were made at least 24 h prior to use. The blood microdialysis probe was positioned within the jugular vein in the direction of right atrium and then perfused with anticoagulant citrate dextrose (ACD) solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow rate of 2 μl/min using the CMA microinjection pump. For brain microdialysis, the rat was mounted on a stereotaxic frame and perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^{2+}; 4 mM K^+; pH 7.0). After being washed with Ringer's solution at a flow-rate of 2 μl/min, the microdialysis probe was implanted in the right hippocampus (coordinates: 5.6 mm posterior to bregma, 5.0 mm lateral to midline and 7.0 mm lower to tip) according to the Paxinos and Watson atlas [18]. The positions of the probes were verified by standard histological procedure at the end of experiments. This method has been previously reported.

2.5. Drug administration

After a 2 h post-implantation period, an intravenous dose of drug was administered via the femoral vein. A vincamine dose of 10 or 30 mg/kg ($n = 6$) was administered intravenously to control group rats. The warm normal saline and 1 M citric acid (80:20, v/v, pH 3.5) was used as injection solvent. For the cyclosporine treated group ($n = 6$), cyclosporine, 10 mg/kg, was injected via femoral vein 10 min prior to vincamine. The injectable concentration of cyclosporine (10 mg/ml) was produced by 5% dextrose/water solution. The total volume of each injection was 1 ml/kg.

The blood was connected to an on-line injector (CMA/160) for direct assay. The brain dialysate was connected a fraction collector (CMA/140) for off-line assay. The sampling interval was 10 min for each probe. Blood and brain dialysates were measured by HPLC on the same day as the experiment.

2.6. Recovery of microdialysate

For in vivo recovery, the blood and brain microdialysis probes were inserted into the jugular vein and brain hippocampus under anaesthesia with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.). Anticoagulant solution containing vincamine (1, 5 and 10 μg/ml) was passed through the microdialysis probe into the rat blood and brain at a constant flow rate (2 μl/min) using an infusion pump (CMA/100). Two hours after probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of vincamine were determined by HPLC. The relative recovery (R_{dial}), in vivo, of vincamine across the microdialysis probes was calculated according to the following equation, $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$.

2.7. Pharmacokinetic application

Vincamine microdialysate concentrations (C_m) were converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic calculation software WinNonlin Standard Edition Version 1.1 (Pharsight, Mountain View, CA, USA) by the compartmental method. The area under the concentration curves (AUCs) from time zero to time infinity were calculated by the trapezoidal rule and extrapolated to time infinity by the addition of AUC_{t-inf}. The AUC values were thus given by the sum of the products of the measured concentrations and the collection time interval, plus the residual area, that is: $AUC = AUC_{0-t} + AUC_{t-inf}$.

2.8. Statistics

The results are represented as mean ± standard error of the mean. Statistical analyses were performed with SPSS version 10.0 (SPSS, Chicago, IL, USA). One-way ANOVA was followed by a Dunnett's post-hoc test comparison between the control (vincamine treated alone), and cyclosporine treated groups. All statistical tests were performed at the two-tailed 5% level of significance.

3. Results and discussion

3.1. Chromatography

Peak scanning by the photodiode array detection (Waters 2695 module coupled to 2696 detector) revealed the maxima wavelength of vincamine to be 220 nm. Typical chromatograms of standards containing vincamine are shown in Fig. 2. Separation of vincamine from endogenous chemicals in blood dialysate was achieved in an optimal mobile phase containing 75% of methanol, 25% of water and 1% diethylamine (pH 7.15). The retention time of vincamine was 6.3 min. Mobile phases containing diethylamine as suggested to enhance the peak character of basic analyte by Ishikawa and Shibata [19], can reduce peak tailing, resulting in narrow peaks and improving the resolution of vincamine from the dialysates in the reversed-phase chromatographic application.

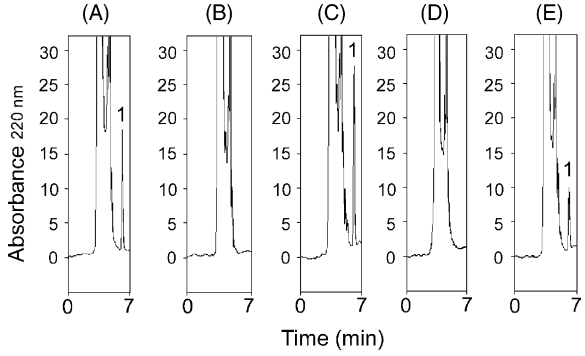

Fig. 2. (A) Standard injection of vincamine (0.5 μg/ml). (B) Chromatogram of a rat blood dialysate sample before vincamine administration. (C) Chromatogram of a rat blood dialysate containing vincamine (0.65 μg/ml) collected 20–30 min after vincamine administration (30 mg/kg, i.v.). (D) Chromatogram of a blank brain hippocampus dialysate. (E) Chromatogram of a brain hippocampus dialysate containing vincamine (0.21 μg/ml) that sample was collected 20–30 min after vincamine administration (30 mg/kg, i.v.). 1, Vincamine.

Fig. 2A shows a standard injection of vincamine (0.5 μg/ml) dissolving in the injection vehicle, whereas Fig. 2B shows the chromatogram of a rat blood dialysate sample before vincamine administration and Fig. 2C shows the chromatogram of a rat blood dialysate containing vincamine (0.65 μg/ml) collected 20–30 min after vincamine administration (30 mg/kg, i.v.). Fig. 2D shows the chromatogram of a blank brain hippocampus dialysate. Fig. 2E shows the chromatogram of a brain hippocampus dialysate containing vincamine (0.21 μg/ml) from a sample that was collected 20–30 min after vincamine administration (30 mg/kg, i.v.).

3.2. Linearity

Linear least-square regression analysis of the calibration graph on 6 different days demonstrated linearity between the response and the nominal concentration of vincamine over the range of 0.05–100 μg/ml. The results of linear regression analysis show that the correlation coefficients of all standards curves were better than 0.995. The data show the excellent reproducibility of the sample analysis.

Table 1
Intra- and inter-assay precision (RSD) and accuracy (Bias) of the HPLC method for the determination of vincamine

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	RSD (%)	Bias (%)
Intra-assay			
0.05	0.054 ± 0.003	6.0	8.0
0.10	0.106 ± 0.004	4.0	6.0
0.50	0.49 ± 0.02	4.1	−2.0
1.00	0.98 ± 0.03	3.1	−2.0
5.00	5.04 ± 0.03	0.4	0.8
10.0	10.1 ± 0.11	1.1	1.0
20.0	20.2 ± 0.2	1.1	0.8
Inter-assay			
0.05	0.045 ± 0.003	6.7	−10.0
0.10	0.095 ± 0.008	8.4	−5.0
0.50	0.48 ± 0.04	8.3	−4.0
1.00	0.99 ± 0.04	4.0	−1.0
5.00	4.91 ± 0.27	5.5	−1.8
10.0	10.2 ± 0.38	3.7	2.0
20.0	20.3 ± 0.76	3.7	1.5

Data are expressed as means ± SD ($n = 6$).

3.3. Limit of detection

The limit of detection (LOD) of vincamine in rat dialysate was determined to be 0.01 μg/ml at a signal-to-noise ratio of 3. The lower limit of quantitation (LOQ) was 0.05 μg/ml.

3.4. Precision, accuracy and recovery

The intra- and inter-assay precision and accuracy values are presented in Table 1. The overall mean precision, defined by the RSD, ranges from 0.4 to 8.4%. Analytical accuracy, expressed as the percent difference of the mean observed values compared with known concentration varies from −10.0 to 8.0%.

3.5. Recovery

The average in vivo recovery of vincamine in rat blood was 53.7 ± 0.7% at the concentrations of 1, 5, and 10 μg/ml ($n = 6$ for each concentration); and in brain was 17.5 ± 0.8% at the concentrations (n = 6). The in vivo recovery (or dialysis efficiency) can be affected by certain factors, mostly physical in nature, such as temperature and perfusion rate. Also the materials used in the construction of the probe and the final dimensions of the probe can affect dialysis efficiency. Thus, each probe must be calibrated prior to use and all physical components must be kept constant.

3.6. Pharmacokinetic application

The concentration versus time curve of vincamine in rat blood is shown in Figs. 3 and 4, indicating that disposition of vincamine in rat blood has a slower and longer elimination phase. The pharmacokinetic models (one- versus two-compartment) were compared according to the

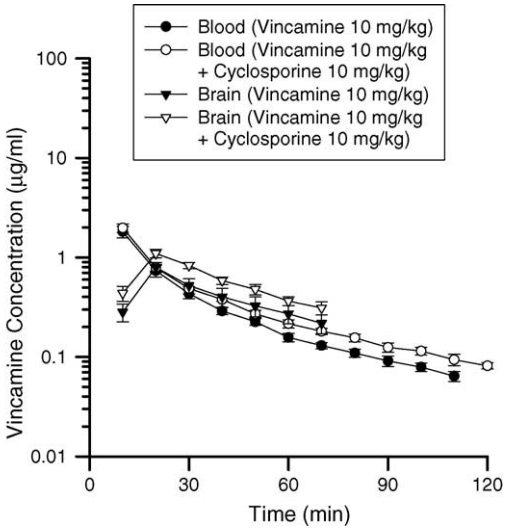

Fig. 3. Mean unbound levels of vincamine in rat blood and brain after vincamine (10 mg/kg, i.v.) administration and co-administration of vincamine (10 mg/kg, i.v.) and cyclosporine (10 mg/kg, i.v.) ($n = 6$). Data are presented as mean ± SEM.

AIC (Akaike Information Criterion) [20], with minimum AIC values being regarded as the best representation of the blood concentration-time course data. A compartmental model with individual animal data after dose was proposed by the computer program WinNonlin. This AIC value in average decreases from −6.09 for one-compartment model to −61.79 for the two-compartment model, indicating that the two-compartment model is more suitable than the one-compartment model for the vincamine administration (30 mg/kg, i.v.). This AIC value on average decreases from 24.0 for one-compartment model to −51.65 for the two-compartment model, indicating that the two-compartment model is better than the one-compartment model for the

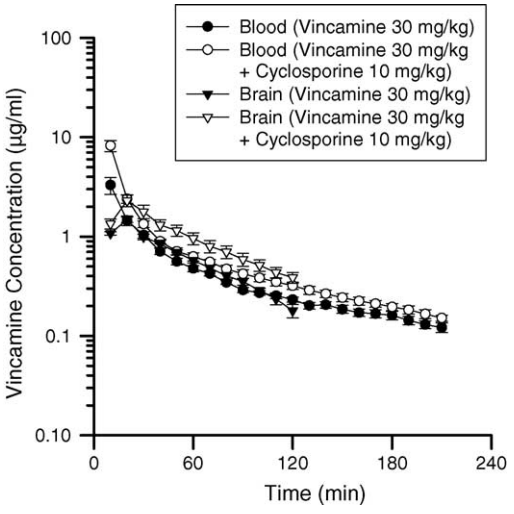

Fig. 4. Mean unbound levels of vincamine in rat blood and brain after vincamine (30 mg/kg, i.v.) administration and co-administration of vincamine (30 mg/kg, i.v.) and cyclosporine (10 mg/kg, i.v.) ($n = 6$). Data are presented as mean ± SEM.

Table 2
Pharmacokinetic parameters of the vincamine administration (10 or 30 mg/kg, i.v.) and the treated group, where cyclosporine 10 mg/kg was injected via femoral vein 10 min prior to vincamine injection

Parameters	10 mg/kg		30 mg/kg	
	Control	Treated	Control	Treated
Blood				
AUC (min μg/ml)	55.1 ± 2.2	80.6 ± 8.3*	157 ± 25.4	379 ± 65.5*
$t_{1/2}$ (min)	32 ± 4	43 ± 4*	56 ± 7	85 ± 19*
C_{max}	2.5 ± 0.3	5.3 ± 1.0*	3.3 ± 0.6	8.2 ± 1.1*
Brain				
AUC (min μg/ml)	35.0 ± 5.3	61.2 ± 6.1*	85.6 ± 4.6	156.4 ± 19*
$t_{1/2}$ (min)	31 ± 6	39 ± 5	36 ± 6	57 ± 6*
C_{max}	0.8 ± 0.1	1.2 ± 0.1*	1.5 ± 0.1	2.3 ± 0.3*
T_{max}	20 ± 0	20 ± 0	20 ± 0	20 ± 0
AUC_{brain}/AUC_{blood}	0.63 ± 0.07	0.76 ± 0.07	0.54 ± 0.20	0.41 ± 0.10

Data are expressed as mean ± SEM ($n = 6$). Significant difference from the values of control group.
* $P < 0.05$.

co-administration of vincamine (30 mg/kg, i.v.) and cyclosporine (10 mg/kg, i.v.). However, Millart et al. suggested that one-compartment model was suitable for vincamine in human subjects [21]. This phenomenon may cause by the diverse assay system and subject difference.

The following equation applies to a two-compartment pharmacokinetic model: $C = Ae^{-\alpha t} + Be^{-\beta t}$; where A and B are the concentration (C) intercept for fast and slow disposition phases, respectively; and α and β are disposition rate constants for fast and slow disposition phases, respectively. Analysis of data after i.v. injection of vincamine at 30 mg/kg and cyclosporine co-administered group yields the following equation $C = 14.08e^{-0.12t} + 0.81e^{-0.01t}$ and $C = 54.86e^{-0.16t} + 1.18e^{-0.01t}$, respectively. The pharmacokinetic parameters as derived from these data and calculated by WinNonlin program are shown in Table 2.

The results of the present experiments suggest that vincamine may penetrate the BBB, in agreement with the earlier observation by Ritschel and Agrawala [11]. The concentration versus time curve of vincamine in blood and brain at dosages of 10, and 30 mg/kg are shown in Figs. 3 and 4. The pharmacokinetic profiles indicate that cyclosporine treated animals significantly increased vincamine level in both blood and brain. Further analysis, the distribution ratio of brain-to-blood (AUC_{brain}/AUC_{blood}) was compensated in the vincamine alone and cyclosporine treated groups (Table 2).

Other methods for measuring vincamine concentration in human plasma have been described using liquid-liquid extraction with *tert*-butyl methyl ether back-extracted into 0.017 M orthophosphoric acid [8]. However, such samples may have to go through a relatively complicated process of sample cleanup before they can be analyzed. By applying the microdialysis technique to a small animal study, the number of animals needed can be substantially reduced since the technique involves a sampling procedure which does not incur large body fluid losses and therefore does not disturb blood homeostasis in the subject.

In conclusion, we have developed a specific and rapid HPLC method for the determination of protein-unbound vincamine in rat blood and brain. This method exhibits no endogenous interference and its sensitivity is sufficient for the determination of biological samples. Results using this technique prove that vincamine is distributed into the brain. Current data obtained from rats show significant impact of cyclosporine on the pharmacokinetics of vincamine in rat blood and brain when they are concomitantly injected. The results indicate that the P-glycoprotein may involve the brain distribution of vincamine.

Acknowledgements

This study was supported in part by research grants (VGH91-377-4D; VGH92-377-4D) from the Veterans General Hospital, Taipei; and (NSC93-2113-M-077-005; NSC93-2320-B-077-006) from the National Science Council, Taiwan.

References

[1] J. Tesseris, G. Roggen, A. Caracalos, D. Triandafillou, Eur. Neurol. 13 (1975) 195.
[2] P. Sprumont, J. Lintermans, Arch. Int. Pharmacodyn. Ther. 237 (1979) 42.
[3] M.E. Karacsony, I. Gyenes, C. Lorincz, Acta Pharm. Hungarica 35 (1965) 280.
[4] V. Vigano, S. Paracchini, G. Piacenza, E. Pesce, Farmaco 33 (1978) 583.
[5] M.R. Smyth, Analyst 111 (1986) 851.
[6] H. Kinsun, M.A. Moulin, J Chromatogr. 144 (1977) 123.
[7] Y. Michotte, D.L. Massart, J. Chromatogr. 344 (1985) 367.
[8] L. Dal Bo, G. Ceriani, G. Broccali, J. Chromatogr. 573 (1992) 158.
[9] C. Dubruc, H. Caqueret, G. Bianchetti, J. Chromatogr. 204 (1981) 335.
[10] S. Caccamese, G. Principato, J. Chromatogr. A 893 (2000) 47.
[11] W.A. Ritschel, P. Agrawala, Meth. Find. Exp. Clin. Pharmacol. 7 (1985) 129.
[12] T.H. Tsai, J. Chromatogr. B 797 (2003) 161.

[13] E.C.M. de Lange, M. Danhof, A.G. de Boer, D.D. Breimer, Brain Res. Rev. 25 (1997) 27.
[14] T. Zetterstrom, T. Etterstrom, T. Sharp, C.A. Marsden, U. Ungerstedt, J. Neurochem. 41 (1983) 1769.
[15] U. Tossman, U. Ungerstedt, Acta Physiol. Scand. 128 (1986) 9.
[16] T.H. Tsai, Br. J. Pharmacol. 132 (2001) 1310.
[17] T.H. Tsai, C.H. Lee, P.H. Yeh, Br. J. Pharmacol. 134 (2001) 1245.
[18] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, CA, 1986.
[19] A. Ishikawa, T. Shibata, J. Liq. Chromatogr. 16 (1993) 859.
[20] K. Yamaoka, T. Nakagawa, T. Uno, J. Pharmacokin. Biopharm. 6 (1978) 165.
[21] H. Millart, D. Lamiable, G. Houin, M. Plat, H. Choisy, J.P. Tillement, Int. J. Clin. Pharmacol. Ther. Toxicol. 21 (1983) 581.