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Determination and pharmacokinetic profile of omeprazole in rat blood, brain and bile by microdialysis and high-performance liquid chromatography

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Abstract

The disposition and biliary excretion of omeprazole was investigated following i.v. administration to rats at 10 mg/kg. We used a microdialysis technique coupled to a validated microbore HPLC system to monitor the levels of protein-unbound omeprazole in rat blood, brain and bile, constructing the relationship of the time course of the presence of omeprazole. Microdialysis probes were simultaneously inserted into the jugular vein toward right atrium, the brain striatum and the bile duct of the male Sprague–Dawley rats for biological fluid sampling after the administration of omeprazole (10 mg/kg) through the femoral vein. The concentration–response relationship from the present method indicated linearity ($r^2>0.995$) over a concentration range of 0.01–50 μg/ml for omeprazole. Intra-assay and inter-assay precision and accuracy of omeprazole fell well within the predefined limits of acceptability. Following omeprazole administration, the blood-to-brain coefficient of distribution was 0.15, which was calculated as the area under the concentration versus time curve (AUC) in the brain divided by the AUC in blood ($k=AUC_{brain}/AUC_{blood}$). The blood-to-bile coefficient of distribution ($k=AUC_{bile}/AUC_{blood}$) was 0.58. The decline of unbound omeprazole in the brain striatum, blood and bile fluid suggests that there was rapid exchange and equilibration between the compartments of the peripheral and central nervous systems. In addition, the results indicated that omeprazole was able to penetrate the blood–brain barrier and undergo hepatobiliary excretion.

Keywords: Microdialysis; Omeprazole; Enzyme inhibitors

1. Introduction

Omeprazole is an acid pump (H^+/K^+-ATPase) inhibitor, which has widely been used against ulcers, gastritis caused by *Helicobacter pylori*, and other acid-related diseases [1]. The H^+/K^+-ATPase, recognized as the gastric proton pump, is involved in the acid secretory process [2]. Omeprazole has been reported to heal duodenal ulcers rapidly [3] and is also used in the treatment of Zollinger–Ellison syndrome [4]. The pharmacokinetics of omeprazole has been studied by radiolabled [^{14}C]omeprazole in rats, dogs and humans [5–8]. Because little central nerve system effect has been reported we, therefore, are interested in its penetration into the brain [9,10].

The characteristics of the microdialysis technique make it a valuable addition to the classical techniques used in pharmacokinetic studies in the central nervous system [11,12]. Over the past several years,

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microdialysis has been increasingly used for in vivo sampling of unbound endogenous or exogenous compounds in the blood, brain, bile or liver in various animal experiments [13–18]. Sampling by this technique involves continuous perfusion of fluid through microdialysis probes implanted in the tissue space which is being dialyzed [19,20]. The aim of this current investigation is to use the microdialysis technique to study the disposition and biliary excretion of omeprazole after a single i.v. dose administration in rats. To achieve this purpose, three microdialysis probes were simultaneously inserted into a rat for sampling biological fluids in the blood, brain and bile.

2. Experimental

2.1. Chemicals and reagents

Omeprazole sodium (Losec) for intravenous infusion solution was purchased from Astra (Södertälje, Sweden). Solvents and reagents of iquid chromatographic grade 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. The animals had free access to food (Laboratory rodent diet 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time only food was removed. Six Sprague–Dawley rats (280–320 g) were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was exposed for further omeprazole administration (10 mg/kg). The body temperature of rats was maintained at 37°C with a heating pad.

2.3. Liquid chromatography

The HPLC system consisted of a chromatographic pump (PM-80; BAS, West Lafayette, IN, USA), an on-line injector (160; CMA, Stockholm, Sweden) equipped with a 10 μl sample loop and a spectrophotometric detector (Dynamax, Walnut Creek, CA, USA). Omeprazole and dialysate were separated using a reversed-phase C_{18} microbore column (Inertsil-2, 150×1 mm I.D.; particle size 5 μm, GS Sciences, Tokyo, Japan) maintained at ambient temperature. The mobile phase was comprised of acetonitrile–20 m*M* monosodium phosphate (pH 7.0) (35:65, v/v) and 0.1 m*M* 1-octanesulfonic acid, and the flow-rate of the mobile phase was 0.05 ml/min. The buffer was filtered through a Millipore 0.22 μm filter and degassed prior to use. The optimal UV detection for omeprazole was done at the wavelength of 300 nm. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

All calibration curves were required to have a correlation value of at least 0.995. The intra-assay and inter-assay variabilities were determined by quantitating six replicates at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 μg/ml using the HPLC method described above on the same day and six consecutive days, respectively. The accuracy (% bias) was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentrations (C_{obs}) as follows: accuracy (% bias)= $[(C_{nom}-C_{obs})/C_{nom}]\times 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: precision (% RSD)= [standard deviation (SD)/C_{obs}]·100. The same data were used to determine both accuracy and precision.

2.5. Microdialysis experiment

Blood, brain and bile microdialysis systems consisted of a CMA/100 microinjection pump (CMA), microdialysis probes and stereotaxic frame. The dialysis probes for blood (10 mm in length), brain (3 mm in length) and bile (7 cm in length) were made

of silica capillary in a concentric design with their tips covered by dialysis membrane (Spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 13 000, Laguna Hills, CA, USA) [15–17,21]. The blood microdialysis probe was positioned within the jugular vein toward the right atrium and then perfused with anticoagulant citrate dextrose, ACD solution (citric acid 3.5 m*M*; sodium citrate 7.5 m*M*; dextrose 13.6 m*M*) at a flow-rate of 1 μl/min.

The bile duct microdialysis probe was constructed in our own laboratory [15,17] based on the design originally described by Scott and Lunte [13] and Hadwiger et al. [14]. A 7-cm dialysis membrane was inserted into polyethylene tubing (PE-60; 0.76 mm I.D.×1.22 mm O.D., Clay-Adams, NJ, USA). The ends of the dialysis membrane and PE-60 were inserted into silica tubing (40 μm I.D×140 μm O.D., SGE, Australia) and PE-10 (0.28 mm I.D.× 0.61 mm O.D.), respectively. Both ends of the tubing and the union were cemented with epoxy. The epoxy was allowed to dry at least for 24 h. For post bile duct cannulation, the microdialysis probe was then perfused with Ringer's solution at 1 μl/min flow-rate.

After the implantation of blood and bile microdialysis probes, the rat was immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The skull was surgically exposed, and a hole was trephined into the skull based on stereotaxic coordinate [22]. The brain microdialysis probe was placed into the right striatum (0.2 mm anterior to bregma, 3.2 mm lateral to midline and 7.5 mm lower to tip). The brain microdialysis probe was perfused with Ringer's solution (147 m*M* Na^+; 2.2 m*M* Ca^{2+}; 4 m*M* K^+; pH 7.0) at a flow-rate of 1 μl/min. Brain dialysates were collected by a fraction collector (CMA/140) at 10 min intervals. The position of each brain microdialysis probe was verified at the end of the experiments [23–25].

2.6. Drug administration

After a 2 h post-surgical stabilization period subsequent to the implantation of probes, omeprazole (10 mg/kg, i.v.) was administered via the femoral vein. The volume of each injection was 1 ml/kg. The dialysates from the blood, brain and bile were connected into an on-line injector (CMA 160) and two fraction collectors (CMA 140), respectively. The sampling interval was 10 min for each probe. Blood, brain and bile dialysates were immediately measured by a validated microbore HPLC system.

2.7. Recovery of microdialysate

For in vivo recovery, the blood, brain and bile microdialysis probes were inserted into the rat jugular vein, brain striatum and bile duct under anesthesia with sodium pentobarbital. Ringer's solutions containing omeprazole 1, 0.5 and 5 μg/ml were passed through the microdialysis probe into rat blood, brain and bile, respectively, at constant flow-rate 1 μl/min using an infusion pump (CMA 100). Following a stabilization period of 2 h post probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of omeprazole were determined by HPLC. The in vivo relative recovery (R_{dial}) of omeprazole across the microdialysis probe was calculated by the following equation [26]: $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$.

2.8. Pharmacokinetic application

Calculated pharmacokinetic values were obtained by treating the individual sets of data. Omeprazole microdialysate concentrations (C_m) were converted to unbound concentration (C_u) as follows [26]: $C_u = C_m/R_{dial}$. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic software WinNonlin Standard Edition Version 1.1 (Scientific Consulting, Apex, NC, USA) by noncompartmental method. The area under the concentration curves (AUC), the area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated using statistical moments [27]. Formation rate constants were calculated from the extrapolated formation slope determined by the method of residuals. The AUCs 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 at the collection time interval, plus the residual area. That is, $AUC = AUC_{0-t} + AUC_{t-inf}$. An analogous method was used for the calculation of the area under the first moment

curve (AUMC) by using the concentration vs. time data. Mean residence time (MRT) was calculated as AUMC/AUC.

3. Results

Typical chromatograms of omeprazole are shown in Fig. 1. Separation of omeprazole from some endogenous chemicals in the blood dialysate was achieved in an optimal mobile phase containing 65% of 20 m*M* monosodium phosphate (pH 7.0), 35% of acetonitrile and 0.1 m*M* 1-octanesulfonic acid. Retention time of omeprazole was 5.8 min. The calibration curve of omeprazole was obtained prior to LC analysis of dialysates over concentration ranges of 0.01–50 μg/ml. Fig. 1A shows a standard injection of omeprazole (0.5 μg/ml), and Fig. 1B shows the chromatogram of a blank blood dialysate. None of the observed peaks interfered with the analyte. Fig. 1C shows the chromatogram of a blood dialysate sample containing omeprazole (1.03 μg/ml) collected from a rat blood microdialysis probe 10 min after omeprazole administration (10 mg/kg, i.v.).

Furthermore, none of the observed peaks interfered with the analyte in the chromatogram of brain sample. Fig. 2A shows a standard injection of omeprazole (0.1 μg/ml), and Fig. 2B shows the chromatogram of a blank brain dialysate. Fig. 2C shows the chromatogram of a brain dialysate sample containing omeprazole (0.034 μg/ml) collected from the rat brain microdialysis probe 10 min after omeprazole administration (10 mg/kg, i.v.).

Fig. 3A shows a standard injection of omeprazole (5 μg/ml). Fig. 3B shows a chromatogram of a blank bile dialysate sample obtained from bile duct microdialysis probe before the drug administration. Fig. 3C shows the chromatogram of bile dialysate sample obtained omeprazole (1.93 μg/ml) collected from the bile duct microdialysis probe 20 min after omeprazole administration (10 mg/kg, i.v.).

The calibration curve of omeprazole was obtained

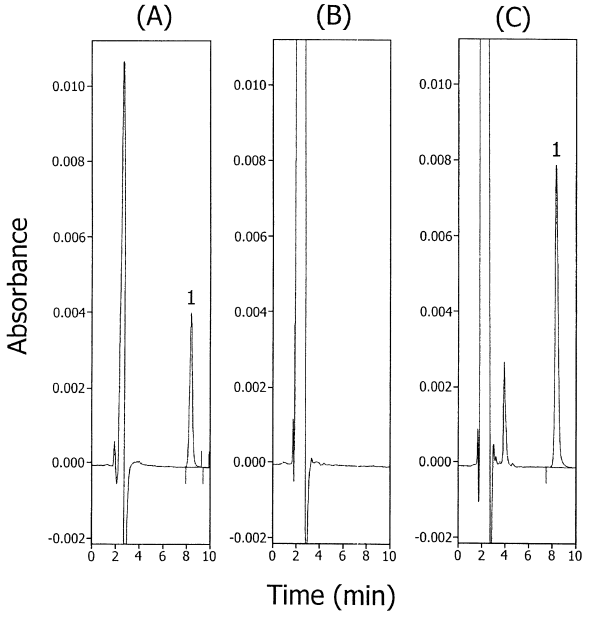

Fig. 1. Typical chromatograms of (A) standard omeprazole (0.5 μg/ml), (B) blank blood dialysate from the microdialysis probe before drug administration, and (C) blood dialysate sample containing omeprazole (1.03 μg/ml) collected from the rat blood microdialysate 10 min post omeprazole administration (10 mg/kg, i.v.). 1: Omeprazole.

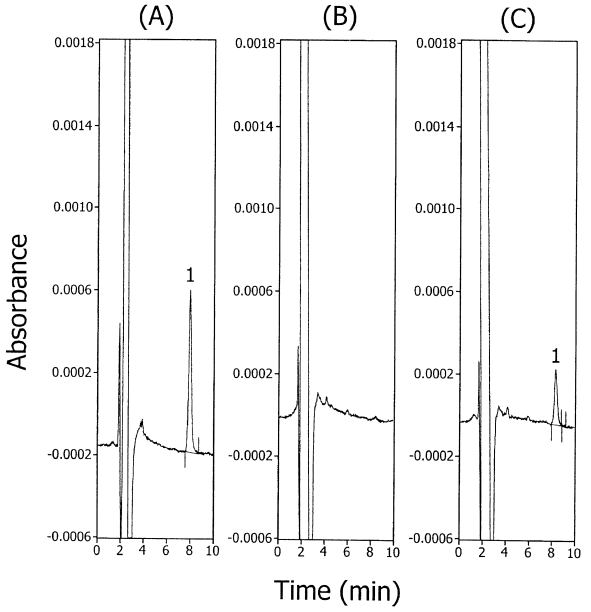

Fig. 2. Typical chromatograms of (A) standard omeprazole (0.1 μg/ml), (B) blank brain dialysate from the microdialysis probe before drug administration, and (C) brain dialysate sample containing omeprazole (0.034 μg/ml) collected from the rat brain microdialysate 10 min post omeprazole administration (10 mg/kg, i.v.). 1: Omeprazole.

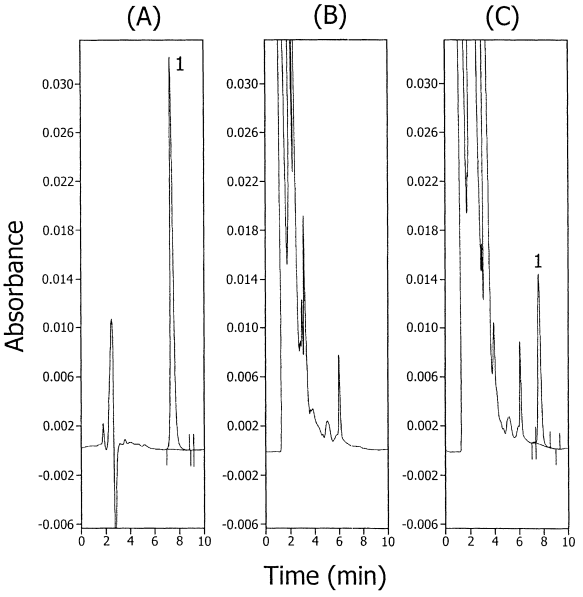

Fig. 3. Typical chromatograms of (A) a standard omeprazole (5 μg/ml), (B) a blank bile dialysate from the flow-through microdialysis probe before drug administration, and (C) a bile dialysate sample containing omeprazole (1.93 μg/ml) collected from rat bile microdialysate 20 min after omeprazole administration (10 mg/kg, i.v.). 1: Omeprazole.

prior to LC analysis of dialysates over a concentration range of 0.01–50 μg/ml. The concentration of omeprazole was linearly related to peak areas of the chromatogram (r^2>0.995). The intra-assay and inter-assay accuracy and precision were thus found to be acceptable for the analysis of a dialysis sample in support of pharmacokinetic studies [28]. As shown in Table 1, the overall mean precision, defined by the RSD, ranged from 0.04 to 9.6%. Analytical accuracy, expressed as the percent difference of the mean observed values compared with known concentration varied from −1.0 to 10.0%. In-vivo recovery of omeprazole in blood (1 μg/ml) was 37.5±2.7% (n=6), in brain (0.5 μg/ml) was 10.5±5.5% (n=6), and in bile (5 μg/ml) was 86.0±1.6% (n=6) (Table 2).

The concentration versus time curves of omeprazole in rat blood, brain and bile are shown in Fig. 4. The above pharmacokinetic curves reflect the fact that the disposition of omeprazole in rat bile exhibited a peak concentration after 20 min of omeprazole administration (10 mg/kg), followed by a slow elimination phase. The AUCs of omeprazole in blood, brain and bile were 73.87±10.33 min μg/ml, 11.34±1.05 min μg/ml and 42.91±5.52 min μg/ml, respectively (Table 3). These results suggest that omeprazole may be excreted from blood to bile and that it penetrates the blood–brain barrier.

Table 1
Intra-assay and inter-assay precision (% RSD) and accuracy (% bias) of the HPLC method for the determination of omeprazole[a]

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	RSD (%)	Bias (%)
Intra-assay			
0.01	0.011±0.001	9.1	10.0
0.05	0.052±0.005	9.6	4.0
0.10	0.099±0.006	6.1	−1.0
0.50	0.501±0.015	3.0	0.2
1.00	1.001±0.008	0.8	0.1
5.00	4.983±0.276	5.5	−0.3
10.00	10.092±0.375	3.7	0.9
50.00	49.988±0.080	0.2	−0.02
Inter-assay			
0.01	0.011±0.001	9.1	10.0
0.05	0.054±0.005	9.3	8.0
0.10	0.101±0.001	1.0	1.0
0.50	0.493±0.012	2.4	1.4
1.00	1.003±0.005	0.5	0.3
5.00	5.063±0.040	0.8	1.3
10.00	10.058±0.104	1.0	0.6
50.00	49.983±0.021	0.04	0.03

[a] Data are expressed as means±S.E.M. (n=6).

4. Discussion

Omeprazole is an ampholyte compound with pK_a

Table 2
In vivo microdialysate recoveries (%) of omeprazole from rat blood, brain and bile[a]

Concentration (μg/ml)	Recovery (%)
In rat blood	
1	37.5±2.7
In rat brain	
0.5	10.5±5.5
In rat bile	
5	86.0±1.6

[a] Data are expressed as mean±SD (n=6).

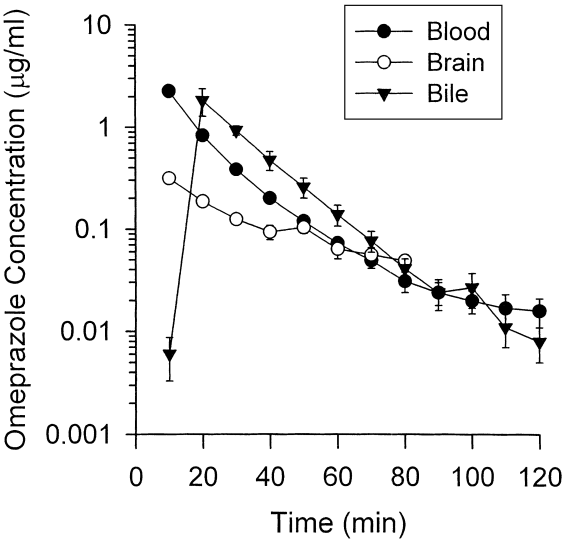

Fig. 4. Mean unbound levels of omeprazole in rat blood, brain and bile after omeprazole administration (10 mg/kg, i.v.; $n=6$).

values of 3.97 (pyridine) and 8.8 (benzimidazole). This causes omeprazole to rapidly degrade in acidic solutions, although it is stable in neutral and alkaline solutions [29,30]. To avoid the degradation of omeprazole during this study, the dialysate samples were immediately transmitted into the chromatographic system by on-line and off-line injectors, followed by the animal experiment [31–33]. The detection limit of omeprazole, at a signal-to-noise ratio of 3, was 10 ng/ml, which was 1/10 less than that described in the previous report [34], but similar to the value reported by Macek et al. [35]. This microdialysis technique coupled to microbore HPLC system is sufficiently sensitive to allow the measurement of unbound omeprazole in rat blood, brain and bile for pharmacokinetic studies. The in-vivo recovery (or dialysis efficiency) can be affected by certain factors, mostly physical in nature, such as temperature and perfusion rate, as well as materials and dimensions of the probe. Thus, each probe must be calibrated prior to use and all physical constants must be kept constant.

Table 3
Pharmacokinetic parameters of omeprazole administration (10 mg/kg, i.v., $n=6$)[a]

Parameters	Estimated
Blood	
AUC (min μg/ml)	73.87±10.33
MRT (min)	12.79±1.92
Brain	
AUC (min μg/ml)	11.34±1.05
MRT (min)	42.55±3.22
Bile	
AUC (min μg/ml)	42.91±5.52
MRT (min)	32.17±3.36
AUC_{brain}/AUC_{blood}	0.15
AUC_{bile}/AUC_{blood}	0.58

[a] Data are expressed as mean±S.E.M. ($n=6$).

The brain penetration of omeprazole, defined as the blood-to-brain coefficient of distribution (k value), was calculated as the omeprazole AUC in brain divided by the omeprazole AUC in blood ($k=AUC_{brain}/AUC_{blood}$) [36]. The k value of unbound omeprazole in brain following omeprazole administration (10 mg/kg, i.v.) was 0.15 (11.34/73.87). omeprazole rapidly diffused into the striatum and blood concentration declined in parallel, suggesting rapid equilibration between the compartment of peripheral and brain. In addition, omeprazole has been reported to significantly decrease cerebrospinal fluid production following i.v. injection [9] and ventriculocisternal perfusion [10]. These results also suggest that omeprazole may penetrate blood–brain barrier, as reflected by the pharmacodynamic observations in the brain [37].

The blood-to-bile coefficient of distribution ($k=AUC_{bile}/AUC_{blood}$) of omeprazole was 0.58 (42.91/73.87). The concentration versus time curve is shown in Fig. 4, and the pharmacokinetic results indicate that the disposition of omeprazole in rat bile shows a slow elimination phase and a peak concentration after 20 min of omeprazole administration (10 mg/kg, i.v.). The results indicate that omeprazole was eliminated via hepatobiliary excretion. Lind et al. [8] using a radiolabeled omeprazole reported that the fecal excretion of omeprazole in humans is entirely accounted for by biliary excretion. The results also agree with Hoffmann et al. [38], who reported that biliary excretion was the major route of elimination of omeprazole metabolites.

Pharmacokinetic studies indicate that omeprazole may cause drug-drug interaction with roxithromycin [39], enteric-coated salicylate [40] and phenytoin [41]. These pharmacokinetic interactions may be

affected by the protein binding [42]. The current results are developed by a protein-unbound assay method for the determination of omeprazole in rat blood, brain and bile. The microdialysis technique provides protein-free samples that can be directly injected into a liquid chromatographic system for continuous in-vivo monitoring of unbound drugs in blood, other biological fluids and tissues [43,44,36]. Compared to the microdialysis techniques, other assay methods which extract drugs from biological samples by silica gel solid-phase extraction [34] and liquid–liquid extraction [35] may have to go through a relatively complicated process of biological sample cleanup before they can be analyzed. The microdialysis technique offers many advantages such as continuous monitoring of analyte concentrations in the extracellular compartment of the same animal, less biological fluid loss and, therefore, minimal stress on hemodynamics [43,44,36].

In conclusion, we have developed a specific, rapid and cost-saving microbore HPLC method for the determination of protein-unbound omeprazole in rat blood, brain and bile. This method exhibits no endogenous interference and its sensitivity is sufficient for the determination of biological samples. Current data obtained from the rat blood, brain and bile firmly suggest that omeprazole penetrates the blood–brain barrier and is excreted in the bile.

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