Journal of Chromatography B, 923–924 (2013) 29–36

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Evaluation of the effect of TM208 on the activity of five cytochrome P450 enzymes using on-line solid-phase extraction HPLC–DAD: A cocktail approach

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ARTICLE INFO

Article history:
Received 23 October 2012
Accepted 16 January 2013
Available online 10 February 2013

Keywords:
Probe drug cocktail
Online solid-phase extraction
HPLC
TM208
Rat plasma

ABSTRACT

A rapid, simple, and sensitive on-line solid-phase extraction HPLC–DAD method for simultaneous evaluation of the activity of five CYP450 isoforms (CYP1A2, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) *in vivo* has been developed and validated. The five specific probe substrates include caffeine (1A2), metoprolol (2D6), dapsone (3A4), omeprazole (2C19) and chlorzoxazone (2E1). Automated pre-purification of plasma and enrichment of analytes were performed using a C18 on-line solid-phase extraction cartridge. After being eluted from the cartridge, the analytes and the internal standard antipyrine were separated on a C18 RP analytical column and analyzed by DAD. The method was validated to quantify the concentration ranges of 0.05–50.0 μg/ml for dapsone and omeprazole, 0.1–50.0 μg/ml for caffeine and 0.2–50.0 μg/ml for metoprolol and chlorzoxazone. The linearity (R^2) for all analytes tested was exceeded 0.99. The intra-day precision ranged from 0.29 to 13% and the inter-day precision ranged from 5.0 to 15%, respectively. The intra-day and inter-day accuracy were between 86.7% and 113.6%. The extraction recoveries were in the range 82.8–109.9% for all the analytes and internal standard antipyrine. This method was successfully applied to evaluate the effects of TM208 on rat five CYP450 isoforms.

1. Introduction

The cytochrome P450 (CYP450) enzymes are a superfamily of hemoproteins that are responsible for approximately 70–80% of the rate-limiting phase I metabolism of drugs [1]. Among the various CYP450 isozymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A are the major isoforms involved in the metabolism of more than 90% of market drugs [2,3]. Although a few pharmaceuticals are metabolized *via* CYP2E1, however, the role of CYP2E1 is without dispute in toxicology [4]. These isozymes display wide interindividual variation, and their activities can be affected by genetic polymorphisms, environmetal factors, dietary components and medicines [5]. Increased CYP450 activities may result in therapeutic failure due to decreased systemic exposure of the substrates, whereas the decreased CYP450 activities may lead to increased systemic exposure, hence increasing the duration or strength of the pharmacodynamic effect [5–8].

Evaluation of the effect of new drugs or drug candidates on CYP450 enzyme activities is essential in pharmaceutical development as it may explain inter-subject variability, investigate potential toxic effects and predict drug–drug interactions [9]. The CYP450 specific probe drugs can be used to determine the real-time activities of important drug-metabolizing enzymes [10,11]. Compared to the administration of single specific probe in multiple studies, the "cocktail" approach can give information on several CYP450 activities of several pathways in a single experiment [12]. Several different cocktails of markers have been used and many cocktail methods have been developed and evaluated in the past years [13–16]. However, the disadvantages of this cocktail approach are also well defined: the frequent occurrence of probe drug side-effects (*in vivo* use), more sample consumption, more time consumption and complicated analytical methods [17]. Nevertheless, the cocktail approach is widely used to assess the activities of CYP450 isoforms and is now one of the basic analytical tools in initial drug evaluation after developing precise analytical methods [18].

The 4-methylpiperazine-1-carbodithioc-acid-3-cyano-3,3-diphenylpropyl ester hydrochloride (TM208) is one of dithiocarbamic acid esters which exhibits significant anticancer activity with low toxicity *in vitro* and *in vivo* [19,20]. Structure

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http://dx.doi.org/10.1016/j.jchromb.2013.01.031

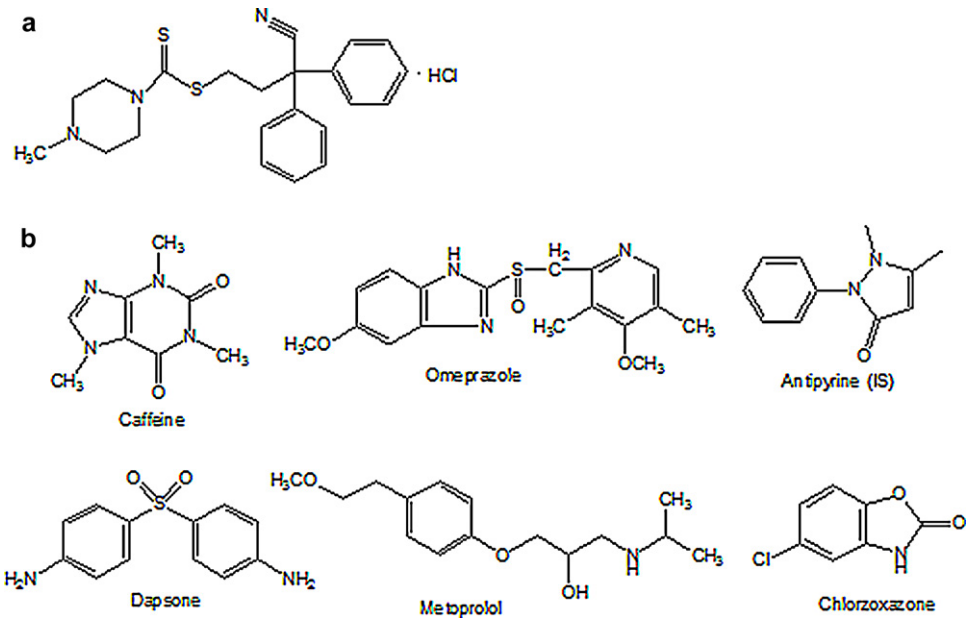

Fig. 1. Structures of (a) TM208, (b) analytes and IS.

of TM208 is shown in Fig. 1a. TM208 and its metabolites were identified in rat plasma, urine, bile, feces, and tissues, and all of them were phase I metabolites [21–25]. In rat liver microsomes, TM208 was mainly metabolized by CYP2D and CYP2B; CYP1A inhibitor had a rather modest inhibitory effect and CYP3A inhibitor seemed to have no inhibitory effect on TM208 metabolism [26]. However, no systematic study has been reported emphasizing the impact of TM208 on CYP450 enzyme activities *in vivo* up to now.

Animal models are commonly used in the preclinical development of new drugs to predict the metabolic behaviour of new compounds in humans, despite the fact that humans differ from animals with regard to isoform composition, expression and catalytic activities of drug-metabolizing enzymes [27]. In this study, rat was chosen as experimental animal.

In this study, an automated high throughput on-line solid-phase extraction (SPE) HPLC–DAD system was used to determine five probe drugs in rat plasma. In comparison with off-line procedures, on-line SPE method can reduce the analysis time, costs for extraction material [28] and labor intensity. Moreover, it can eliminate matrix interference to improve sensitivity and decrease sample loss. The aim of this study was to develop and validate a rapid, simple and sensitive analytical method for simultaneous quantification of caffeine (1A2), metoprolol (2D6), dapsone (3A4), omeprazole (2C19) and chlorzoxazone (2E1) in rat plasma samples, and to apply the developed method to evaluate the effects of TM208 on rat five CYP450 isoforms.

2. Materials and methods

2.1. Chemicals and reagents

Caffeine (CAF, 99.0%), metoprolol (MET, 99.0%), dapsone (DDS, 97.0%), omeprazole (OME, 98.0%), chlorzoxazone (CZX, 98.0%) and antipyrine (ANT, used as IS, 99.0%) were purchased from J&K scientific Ltd. (Beijing, China). Structures of these analytes and IS are shown in Fig. 1b. TM208 (purity > 99%) was synthesized and purified by our research group.

Acetonitrile (HPLC-grade) was purchased from Sayfo Scientific (Tianjin, China). Methanol (isocratic HPLC grade) was purchased from Scharlau Scientific (Spain). Distilled water used in this study was purchased from Wahaha Group (Hangzhou, China).

2.2. Preparation of standard and quality control samples

Stock solution contained CAF, MET, DDS, OME and CZX was prepared at a concentration of 1.0 mg/ml by dissolution in methanol, followed by ultrasonication for 10 min. Then a series of standard solutions (0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 500.0 μg/ml) were prepared in methanol by appropriate dilution of the stock solution. The stock solution of the IS was prepared at 1.0 mg/ml in methanol, followed by ultrasonication for 10 min. Its working solution (20.0 μg/ml) was prepared by appropriate dilution of the stock solution. Once prepared, all the stock solutions were sealed and stored in darkness at 4 °C. Finally, calibration standard samples were prepared at concentrations of 0.05, 0.2, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 μg/ml for DDS and OME, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 μg/ml for CAF and 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 μg/ml for MET and CZX by spiking 10 μl standard solution of corresponding concentrations to 100 μl of blank rat plasma. And, the quality control (QC) samples at three concentration levels (1.0, 5.0 and 20.0 μg/ml) were prepared in the same manner. IS was spiked into the QC samples at a final concentration of 2.0 μg/ml.

2.3. Sample preparation

Frozen plasma samples were taken out from the −20 °C freezer, kept at room temperature for 30 min, allowed to thaw, then adequately vortexed. A total of 100 μl of plasma was transferred into a 1.5 ml centrifuge tube, to which 10 μl of standard working solution of IS (20.0 μg/ml) and 190 μl of methanol were added. The resulting solution was vortexed for 1 min and subsequently centrifuged at 12,000 rpm for 15 min at 4 °C. A total of 200 μl of supernatant was transferred into autosampler vials for analysis.

2.4. Chromatographic systems and conditions

The HPLC instrument (Dionex Ultimate 3000 HPLC system, Dionex, USA) consisted of two ultimate 3000 binary pumps, an ultimate 3000 RS column compartment, an ultimate 3000 thermostat well-plate autosampler injector fitted with a 100 μl sample

Table 1
Gradient elution programs of on-line SPE and HPLC.

Right pump (loading)				Left pump (analytical)			
Time (min)	*B* %[b]	*C* %[a]	Flow (ml/min)	Time (min)	*A* %[a]	*B* %[b]	Flow (ml/min)
0.000	1	99	1.0	0.000	80	20	1.0
2.000	1	99	1.0	3.000	80	20	1.0
2.001	70	30	1.0	3.100	75	25	1.0
6.000	70	30	1.0	5.000	75	25	1.0
8.000	1	99	0.2	6.000	70	30	1.0
16.000	1	99	0.2	7.000	70	30	1.0
18.000	1	99	1.0	10.000	60	40	1.0
				11.000	60	40	1.0
				11.001	5	95	1.0
				15.000	5	95	1.0
				15.001	80	20	1.0
				18.000	80	20	1.0

[a] *A*, *C*%, water with 5 mM ammonium formate.
[b] *B*%, acetonitrile.

loop, an ultimate 3000 DAD, and a column oven incorporating a 6-port switching valve (Dionex, Sunnyvale, CA, USA). The on-line SPE was carried out on an Acclaim 120 C18 cartridge (10 mm × 4.3 mm, 5 μm, i.d.; Dionex, USA). Compounds were separated on a Luna C18 (2) column (150 mm × 4.6 mm, 5 μm, i.d.; Phenomenex, USA). The column temperature was maintained at 30 °C, and the detection wavelengths were 220 and 280 nm. The acquisition time was 18 min. The injection volume was 50 μl. The right-hand pump was used to load the sample from the loop on to the SPE column by a gradient condition from acetonitrile (component B) and water with 5 mM ammonium formate (component C). The left-hand pump was used for the analyzing, and the mobile phase consisted of water with 5 mM ammonium formate (component A) and acetonitrile (component B). The gradient elution programs for both the right (loading) and left (analytical) pumps are shown in Table 1. Beginning transfer time (V1) and ending transfer time (V2) were 2.7 and 3.5 min, respectively. At the state of V1, the 2-position 6-port switching valve was 2–1 and at the state of V2, the switching valve was 6–1. First, the target analytes were selectively extracted from the matrix and preconcentrated on the SPE column; then from V1 to V2, the trapped analytes were transferred in backflush mode to a conventional C18 analytical HPLC column for separation and detection. The schematic diagram of on-line SPE–HPLC–DAD system is shown in Fig. 2.

2.5. Method validation

2.5.1. Selectivity

The chromatographic interference from endogenous materials or other sources was estimated by comparing chromatograms of blank rat plasma from six sources, plasma spiked with CAF, MET, DDS, OME, CZX and IS, and plasma samples obtained from pharmacokinetic studies.

2.5.2. Linearity and sensitivity

The calibration curves for each analyte were constructed using the analyte/IS peak area ratio *versus* the analyte concentration. Microsoft Office Excel was used for linear regression analysis of the calibration data.

The sensitivity of the method was evaluated as the lower limit of quantification (LLOQ). The lower limit of quantification (LLOQ) in plasma was defined as the lowest concentration on the calibration curve for which the signal-to-noise ratio was 10:1.

2.5.3. Precision, accuracy, recovery and stability

The precision, accuracy, recovery and stability of the method were determined by measuring five replicates of QC samples at three concentration levels (20.0, 5.0 and 1.0 μg/ml). The recovery for IS was only measured at one concentration level (2.0 μg/ml).

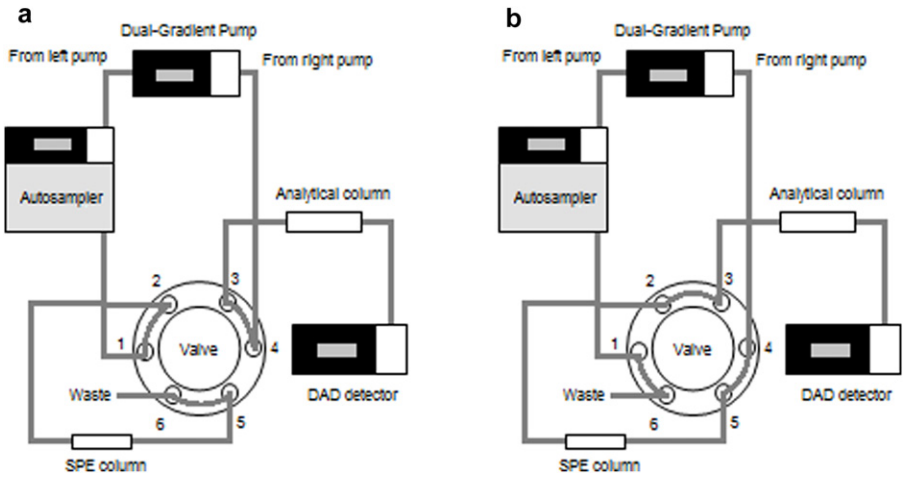

Fig. 2. Instrumental configuration of on-line SPE: (a) at the state of V1, sample was loaded onto the SPE column and (b) at the state of V2, the trapped analytes were transferred in backflush mode to analytical column.

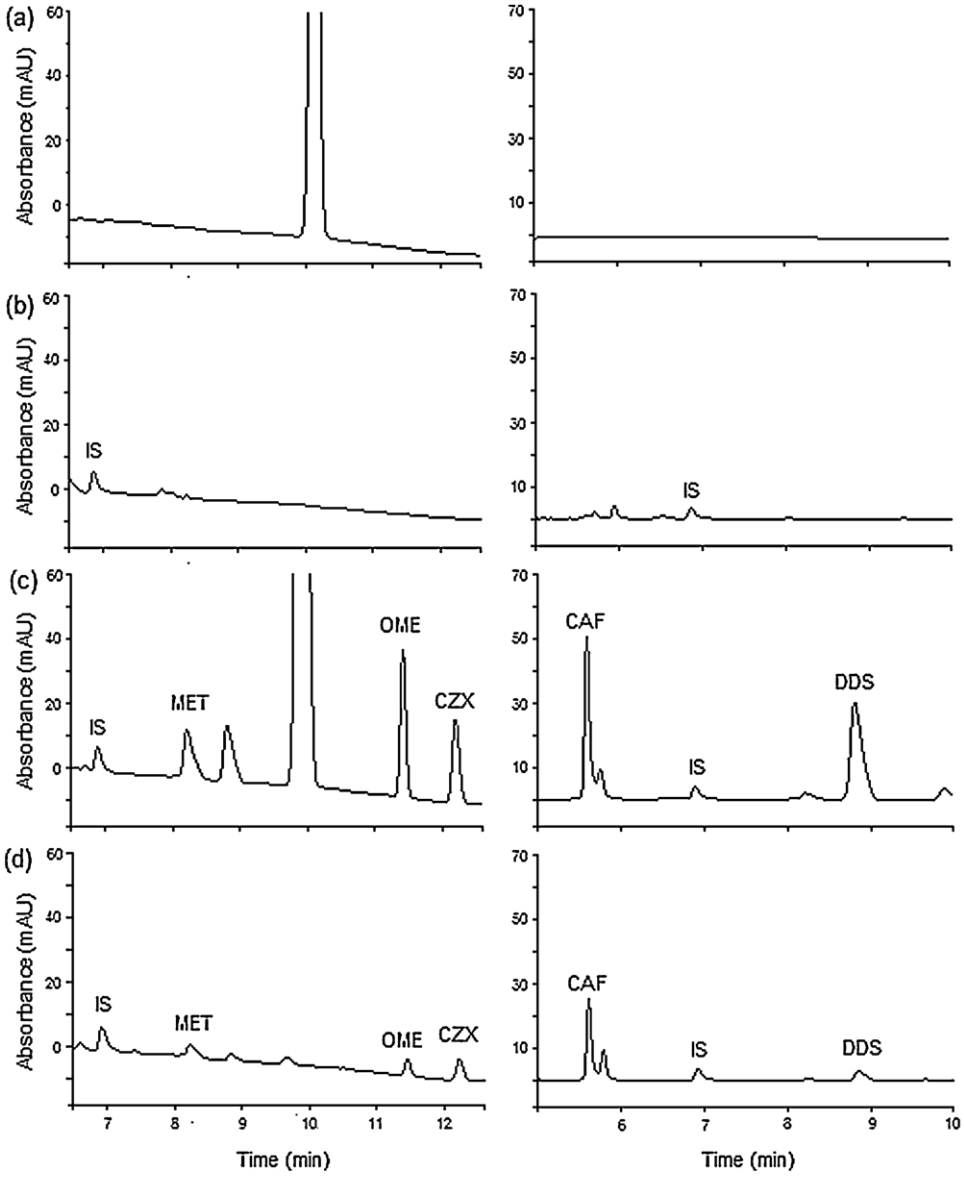

Fig. 3. Representative chromatograms. Detection wavelength: 220 nm (left) and 280 nm (right). (a) Blank plasma sample, (b) blank plasma sample spiked only with IS, (c) blank plasma sample spiked with CAF, MET, DDS, OME, CZX and IS and (d) rat plasma sample 0.25 h after the oral administration of the cocktail solution.

The intra-day precision and inter-day precision were determined by analyzing five replicates of QC samples described above on a single day and five consecutive days, respectively. Accuracy was calculated by dividing the measured mean drug concentration by the theoretical drug concentration. The acceptable criteria of data included accuracy within ±15% bias from the nominal values and a precision within ±15% relative standard deviation (RSD). Extraction recovery was defined as the ratio of analyte peak area from plasma to peak area from the corresponding sample without plasma matrix. The acceptance criterion for the precision of the extraction recovery at each level was ±15% relative standard deviation (RSD). The stability of CAF, MET, DDS, OME and CZX in rat plasma was evaluated by measuring the area ratio response (analyte/IS) of stored samples against the freshly prepared ones. The long-term stability was tested after storage at −20 °C for a week. The short-term stability was evaluated after storage at room temperature (25 °C) for 24 h without light. Freeze-thaw stability of the samples was measured over three freeze-thaw cycles of thawing at standardized temperature (25 °C) for 1 h and refreezing at −20 °C for 24 h. The analytes were considered stable if the values for %change were within ±15% and the precision ≤ 15% was acceptable.

2.6. Application of the method to pharmacokinetic analysis

Male Sprague-Dawley rats weighting 200 ± 20 g were obtained from the Lab Animal Institute of Peking University Health Center (Beijing, China). The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. The animals were acclimatized for at least one week in a standardized temperature (25–28 °C), humidity (50–60%), and light (12 h light/12 h dark) environment with free access to standard food and tap water before initiation of the experimental procedures. Ten rats were randomly divided into TM208 treated group ($n = 5$) and control group ($n = 5$). TM208 was administered orally at a dose of 150.0 mg/kg/day body weight (TM208 solution was prepared by ultrasonic dissolving in normal saline for 20 min) and an equal volume of 0.9% saline alone was administered to the

Table 2
Equations of calibration curve for CAF, MET, DDS, OME and CZX ($n = 5$).

Compound	Calibration range (μg/ml)	Linear regression equation	R^2
Caffeine	0.1–50.0	$Y = 0.7412\,X - 0.1745$	0.9978
Metoprolol	0.2–50.0	$Y = 0.5360\,X - 0.1542$	0.9966
Dapsone	0.05–50.0	$Y = 1.0225\,X + 0.0374$	0.9956
Omeprazole	0.05–50.0	$Y = 0.9230\,X - 0.0112$	0.9973
Chlorzoxazone	0.2–50.0	$Y = 0.6811\,X - 0.0823$	0.9971

control group. Drugs and saline were orally administrated one time for consecutive 7 days. On the 8th day, all rats were administrated orally with the cocktail solution consisted of CAF (20 mg/kg), MET (20 mg/kg), DDS (20 mg/kg), OME (20 mg/kg) and CZX (20 mg/kg). Blood (0.3 ml each time) was collected from the ocular vein into centrifuge tubes at pre-dose (0.0 h) and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose. All blood samples were immediately centrifuged at 12,000 rpm for 15 min at 4 °C and the separated plasma was stored at −20 °C until analysis.

Pharmacokinetic parameters were determined by using a published, free Microsoft Excel add-in, "PKSolver." The specifications of this program as well as validation were published in the January 2010 edition of Computer Methods and Programs in Biomedicine [29].

3. Results and discussion

3.1. On-line solid-phase extraction HPLC

The difficulty in simultaneous analysis of multiple CYP450 substrates in plasma was the low concentration level of CYP450 probe drugs. Therefore, analytical methods with higher specificity and sensitivity are required. Many methods for simultaneous determination of multiple CYP450 substrates in one measurement session with low LODs have been published, but only with the use of MS detection [30]. To overcome this limitation and to increase the sensitivity, preconcentration strategy was applied. By solid-phase extraction process, preconcentration of target compound could be performed concomitantly with sample clean-up and extraction, resulting in an improvement in the specificity and sensitivity [31]. To accelerate the analytical speed, different gradient elution programs were employed for SPE and HPLC system during development of the analytical method. Through an on-line SPE step, rapid enrichment and effective extraction of the target analytes could be attained and the next analytical procedure could be more rapid.

3.2. Method validation

3.2.1. Selectivity

Representative chromatograms of plasma samples are presented in Fig. 3. The retention times of the analytes were 5.6, 8.2, 8.8, 11.4 and 12.2 min for CAF, MET, DDS, OME and CZX, respectively. The retention time of IS was 6.9 min. No significant interference from endogenous materials or other sources was found at the same retention time as the target analytes.

3.2.2. Linearity and sensitivity

Linearity was investigated over the range of 0.05–50.0 μg/ml for DDS and OME, 0.1–50.0 μg/ml for CAF and 0.2–50.0 μg/ml for MET and CZX. The linear regression equations of the detector responses in mAU.s (evaluated by analyte/IS peak area ratio) at 220 nm for MET, OME and CZX, and at 280 nm for CAF and DDS *versus* concentration (μg/ml) are presented in Table 2. The correlation coefficients (R^2) of the curves exceeded 0.99 for each compound.

For sensitivity determination, the lower limit of quantification (LLOQ) for DDS and OME was 50 ng/ml. LLOQ precision was 5.4% for DDS and 6.1% for OME, respectively. The LLOQ for CAF was 100 ng/ml, and for MET and CZX was 200 ng/ml. LLOQ precision was 0.46% for CAF, 6.2% for MET and 5.8% for CZX, respectively.

Table 3
Extraction recoveries, intra- and inter-day precision and accuracy for CAF, MET, DDS, OME and CZX ($n = 5$).

Compound	Nominal conc. (μg/ml)	Intra-day			Inter-day			Extraction recovery (%)	RSD (%)
		Precision		Accuracy[a] (%)	Precision		Accuracy[a] (%)		
		Mean ± SD	RSD (%)		Mean ± SD	RSD (%)			
Caffeine	1.0	0.95 ± 0.09	12.1	94.8	0.92 ± 0.08	10.9	92.4	82.8	6.2
	5.0	5.32 ± 0.01	0.3	106.3	5.56 ± 0.27	5.0	111.2	109.9	1.4
	20.0	17.49 ± 0.13	0.8	87.4	19.01 ± 1.42	7.6	95.0	89.5	1.4
Metoprolol	1.0	1.09 ± 0.10	13.0	109.0	1.09 ± 0.11	13.6	109.0	94.8	2.8
	5.0	5.30 ± 0.08	1.7	106.0	5.06 ± 0.66	13.8	101.3	94.2	2.6
	20.0	18.47 ± 0.55	3.0	92.4	17.33 ± 1.39	8.1	86.7	87.2	1.2
Dapsone	1.0	0.98 ± 0.01	0.7	98.4	0.95 ± 0.07	6.9	94.7	94.9	0.5
	5.0	4.33 ± 0.06	1.4	86.7	5.11 ± 0.58	11.3	102.2	99.1	0.8
	20.0	19.33 ± 0.08	0.4	96.7	19.08 ± 1.43	7.5	95.4	102.5	0.7
Omeprazole	1.0	1.07 ± 0.02	2.1	107.4	1.07 ± 0.16	15.0	107.5	101.4	1.9
	5.0	4.58 ± 0.05	1.0	91.5	5.38 ± 0.49	9.1	107.5	108.3	1.0
	20.0	18.76 ± 0.16	0.8	93.8	20.14 ± 2.33	11.6	100.7	107.3	0.7
Chlorzoxazone	1.0	1.14 ± 0.02	2.3	113.6	1.12 ± 0.14	14.3	112.4	98.3	1.5
	5.0	4.52 ± 0.06	1.4	90.4	5.25 ± 0.41	8.1	104.9	104.2	0.6
	20.0	18.43 ± 0.20	1.1	92.1	19.42 ± 1.76	9.1	97.1	103.7	0.9

[a] Accuracy was calculated by dividing the measured mean drug concentration by the theoretical drug concentration.

Table 4
Stability of CAF, MET, DDS, OME and CZX in rat plasma under various storage conditions ($n = 5$).

Compound	Nominal conc. (μg/ml)	Storage conditions					
		25 °C for 24 h		−20 °C for a week		Freeze/thaw (3 cycles)	
		%Change[a]	RSD (%)	%Change[a]	RSD (%)	%Change[a]	RSD (%)
Caffeine	1.0	3.2	3.1	−1.6	7.1	0.4	5.8
	5.0	10.1	0.6	−8.7	2.3	−6.6	2.5
	20.0	1.3	0.7	−12.0	2.6	−0.7	3.9
Metoprolol	1.0	−16.7	1.1	2.0	13.1	−2.9	11.4
	5.0	−16.2	0.5	3.7	0.4	−14.3	13.4
	20.0	4.8	0.6	5.8	1.2	−2.0	4.7
Dapsone	1.0	3.4	0.7	1.4	3.0	−9.6	2.2
	5.0	−5.4	0.3	−7.2	1.0	−6.8	5.6
	20.0	−0.9	0.6	−4.0	1.0	−0.1	1.6
Omeprazole	1.0	2.5	0.4	−0.7	1.2	−16.0	4.4
	5.0	−1.4	0.7	−5.1	1.4	−3.3	2.9
	20.0	2.3	1.0	−4.8	3.9	5.3	2.9
Chlorzoxazone	1.0	2.0	1.7	2.3	2.0	−4.8	4.3
	5.0	2.2	1.1	−0.2	0.6	−1.0	2.4
	20.0	6.6	0.6	2.6	1.3	3.6	3.3

[a] %Change = (mean stored samples − mean comparison samples/mean comparison samples) × 100.

3.2.3. Precision, accuracy, recovery and stability

The intra-day precision and accuracy were determined by performing five replicates of QC samples at three concentration levels on the same day, the relative intra-day standard deviation (RSD) ranged from 0.29% to 13%. Similarly, the inter-day precision and accuracy were determined by performing five replicates of QC samples at three concentration levels on five different days; in this case, the relative standard deviation (RSD) ranged from 5.0% to 15%. The intra-day and inter-day accuracy ranged from 86.7% to 113.6%. The intra- and inter-day accuracy was within ±15% (85–115%) and precision was also within the acceptable range of 15% for all QC samples for all analytes. The QC data illustrate the stability and reliability of this method. The extraction recovery for all analytes ranged from 82.8% to 109.9% at three concentration levels. Extraction recovery of the IS was 90.6%. The precision and accuracy data derived from analysis of these replicate samples and the extraction recovery data for analytes are shown in Table 3.

The stability of CAF, MET, DDS, OME and CZX was investigated under various storage conditions. All stability results are shown in Table 4. CAF, MET, DDS, OME and CZX were stable (%change within ±15%) without significant degradation under −20 °C storage conditions for a week and five probe drugs in rat plasma could therefore be stored under −20 °C storage conditions for a week. CAF, DDS, OME and CZX were stable (%change within ±15%) at room temperature (25 °C) for 24 h without light. Therefore, the stability experiments suggest that plasma samples should be extracted and analyzed quickly; otherwise, it need to be stored under −20 °C to avoid the risk of analyte degradation and analyzed within one week.

3.3. Application to pharmacokinetic study

The present on-line SPE–HPLC–DAD method was successfully applied to study the pharmacokinetics of five probe drugs in rats. The mean concentration–time curves in rats are depicted in Fig. 4. The main pharmacokinetic parameters of five probe drugs are listed in Table 5. TM208 on rat five CYP450 enzyme activities was evaluated through the pharmacokinetic parameters of probe substrates. Comparisons between two groups were performed by two-tailed *t*-test.

The value of apparent oral clearance (CL/F) ($p < 0.01$) of CZX was significantly different between TM208 treated group and control group, indicating that TM208 may accelerate the clearance of CZX. The mean residence time (MRT) of DDS was significantly different ($p < 0.01$) between TM208 treated group and control group, demonstrating that TM208 may shorten the duration of systemic exposure to DDS. The result suggested that TM208 had a potential interaction (possibly induction) with CYP2E1 and CYP3A4, or some other drug metabolizing enzymes or transporters.

The pharmacokinetic parameters of CAF, OME, MET and in rats showed no significant difference ($p > 0.05$) between TM208 treated group and control group, indicating that TM208 showed no significant influence on rat CYP1A2, CYP2D6, and CYP2C19 activities *in vivo*.

Table 5
Pharmacokinetic parameters of CAF, MET, DDS, OME and CZX after dosing (mean ± SD, $n = 5$).

Parameter	TM208 treatment group	Control group
Caffeine		
C_{max} (μg/ml)	12.40 ± 3.11	12.97 ± 5.58
CL/F (mg)/(μg/ml)/h)	0.06 ± 0.02	0.05 ± 0.01
MRT (h)	4.92 ± 1.29	6.06 ± 0.91
Metoprolol		
C_{max} (μg/ml)	2.26 ± 0.75	1.42 ± 0.78
CL/F (mg)/(μg/ml)/h)	1.27 ± 1.09	0.99 ± 0.36
MRT (h)	3.60 ± 2.18	3.70 ± 1.08
Dapsone		
C_{max} (μg/ml)	3.00 ± 0.95	3.04 ± 1.83
CL/F (mg)/(μg/ml)/h)	0.14 ± 0.04	0.11 ± 0.06
MRT (h)	9.77 ± 1.24**	15.28 ± 2.67
Omeprazole		
C_{max} (μg/ml)	1.36 ± 0.24	0.49 ± 0.58
CL/F (mg)/(μg/ml)/h)	4.61 ± 2.66	4.94 ± 3.67
MRT (h)	1.84 ± 0.74	6.24 ± 4.87
Chlorzoxazone		
C_{max} (μg/ml)	7.88 ± 4.79*	16.76 ± 6.58
CL/F (mg)/(μg/ml)/h)	0.12 ± 0.04**	0.05 ± 0.02
MRT (h)	4.25 ± 0.39	5.77 ± 1.92

C_{max}, the maximum concentration of a drug in the body after dosing; CL/F, apparent total body clearance of drug from plasma; MRT, mean residence time.

* $p < 0.05$ compared with control group by two-tailed *t*-test.

** $p < 0.01$ compared with control group by two-tailed *t*-test.

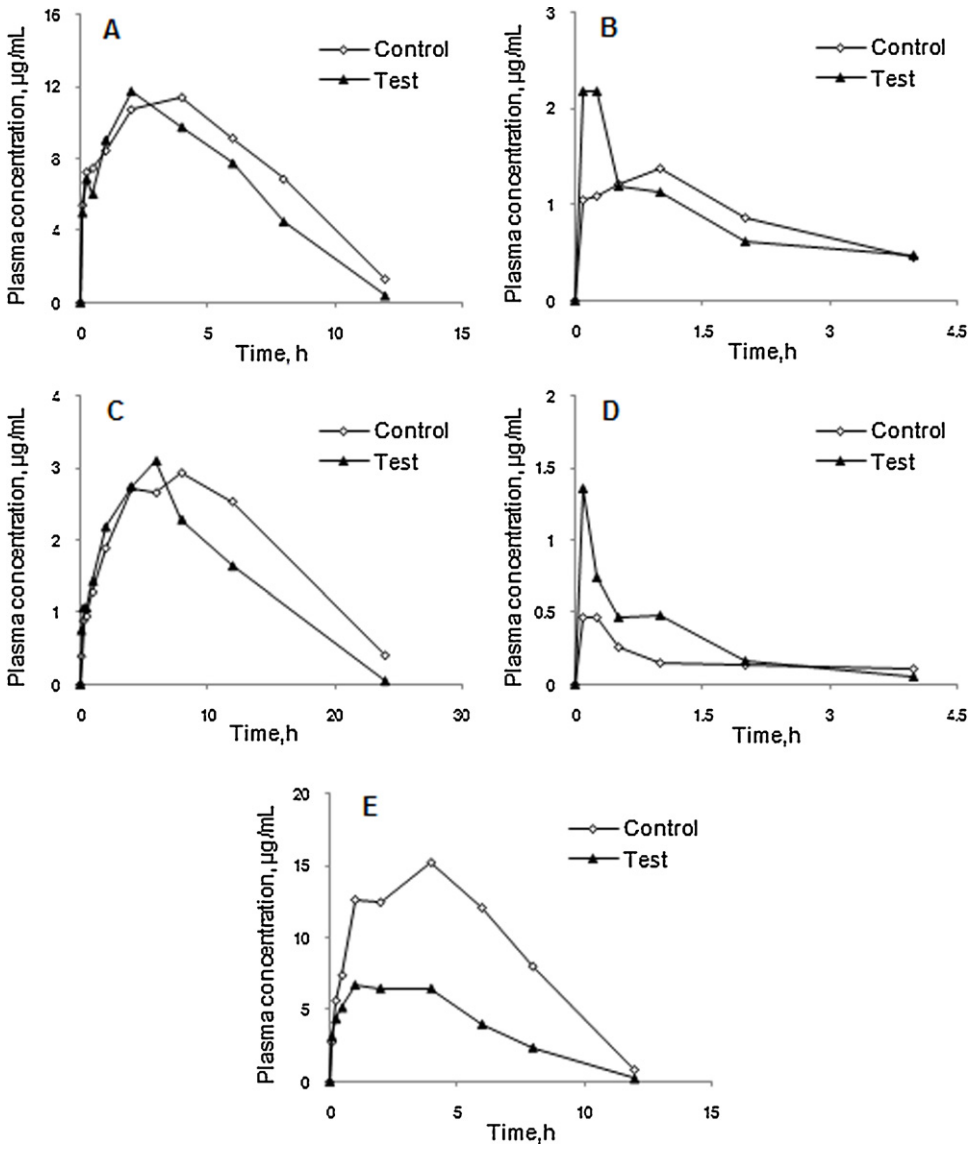

Fig. 4. Mean plasma concentration–time curves of CAF (A), MET (B), DDS (C), OME (D) and CZX (E) after the oral administration of the cocktail solution.

4. Conclusions

A rapid, simple, and sensitive on-line solid-phase extraction HPLC–DAD method has been developed and validated for the determination of caffeine, metoprolol, dapsone, omeprazole and chlorzoxazone. The method was demonstrated to be sensitive and convenient, with short analysis time and low variable costs. The method has been employed to determine the activity of multiple CYP450 isoforms simultaneously and successfully applied to evaluate the effects of TM208 on rat CYP1A2, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. The results demonstrated that TM208 had a potential interaction (possibly induction) with CYP2E1 and CYP3A4, or some other drug metabolizing enzymes or transporters in rats; however, continuous administration of TM208 might not significantly affect CYP1A2, CYP2D6 and CYP2C19-mediated metabolism in rats.

Acknowledgements

This work was financially supported by the National Natural Science Foundation (No. 81072612), the Natural Science Foundation of Beijing (No. 7102107), the Open Foundation of State Key Laboratory of Natural and Biomimetic Drugs (No. K20110109) and Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20110001110021).

References

[1] S. Rendic, Drug Metab. Rev. 34 (2002) 83.
[2] T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, F.P. Guengerich, J. Pharmacol. Exp. Ther. 270 (1994) 414.
[3] G.W. Chang, P.C. Kam, Anaesthesia 54 (1999) 42.
[4] F.J. Gonzalez, Mutat. Res. 569 (2005) 101.
[5] O. Pelkonen, M. Turpeinen, J. Hakkola, P. Honkakoski, J. Hukkanen, H. Raunio, Arch. Toxicol. 82 (2008) 667.
[6] U.M. Zanger, M. Turpeinen, K. Klein, M. Schwab, Anal. Bioanal. Chem. 392 (2008) 1093.
[7] L.W. Wormhoudt, J.N. Commandeur, N.P. Vermeulen, Crit. Rev. Toxicol. 29 (1999) 59.
[8] L. Bertilsson, Clin. Pharmacokinet. 29 (1995) 192.
[9] K. Jiang, K. Li, F. Qin, X. Lu, F. Li, Toxicol. In Vitro 25 (2011) 1033.
[10] K.T. Kivisto, H.K. Kroemer, J. Clin. Pharmacol. 37 (1997) 40S.
[11] D.S. Streetman, J.S. Bertino Jr., A.N. Nafziger, Pharmacogenetics 10 (2000) 187.
[12] D.D. Breimer, Clin. Pharmacokinet. 8 (1983) 371.
[13] S. Zhang, N. Song, Q. Li, H. Fan, C. Liu, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 871 (2008) 78.
[14] A. Kumar, H.J. Mann, R.P. Remmel, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 853 (2007) 287.
[15] A. Tolonen, A. Petsalo, M. Turpeinen, J. Uusitalo, O. Pelkonen, J. Mass Spectrom. 42 (2007) 960.

[16] V. Dixit, N. Hariparsad, P. Desai, J.D. Unadkat, Biopharm. Drug Dispos. 28 (2007) 257.
[17] E. Tanaka, N. Kurata, H. Yasuhara, J. Clin. Pharm. Ther. 28 (2003) 157.
[18] G. Zlokarnik, P.D. Grootenhuis, J.B. Watson, Drug Discov. Today 10 (2005) 1443.
[19] W. Guo, F.X. Ran, R.Q. Wang, J.R. Cui, R.T. Li, T.M. Cheng, Z.M. Ge, Chin. J. Clin. Pharmacol. Ther. 9 (2004) 59.
[20] N. Zhang, W. Guo, L. Wang, W. Huang, B. Xu, Z.M. Ge, M. Li, R.T. Li, J.R. Cui, Anticancer Drugs 19 (2008) 593.
[21] X.M. Jiang, X.M. Ling, R.T. Li, J.R. Cui, Acta Pharmacol. Sin. 41 (2006) 1084.
[22] X.M. Jiang, D.T. Kong, F.B. Han, X.M. Ling, X.Y. Liu, R.T. Li, J.R. Cui, Rapid Commun. Mass Spectrom. 21 (2007) 1599.
[23] X.M. Jiang, X.M. Ling, F.B. Han, R.T. Li, J.R. Cui, J. Sep. Sci. 30 (2007) 2080.
[24] X.M. Jiang, X.M. Ling, F.B. Han, R.T. Li, J.R. Cui, J. Pharm. Biomed. Anal. 44 (2007) 1127.
[25] J. Wang, Y. Song, J.M. Zhang, X.M. Li, X.M. Ling, R.T. Li, J.R. Cui, Chromatographia 72 (2010) 459.
[26] D.T. Kong, X.M. Ling, F.B. Han, J.L. Gong, Z.M. Ge, R.T. Li, J. Chin. Pharm. Sci. 17 (2008) 30.
[27] M. Martignoni, G.M. Groothuis, R. de Kanter, Expert Opin. Drug Metab. Toxicol. 2 (2006) 875.
[28] K. Stoob, H.P. Singer, C.W. Goetz, M. Ruff, S.R. Mueller, J. Chromatogr. A 1097 (2005) 138.
[29] Y. Zhang, M.R. Huo, J.P. Zhou, S.F. Xie, Comput. Methods Progr. Biomed. 99 (2010) 306.
[30] J. Jurica, J. Konecny, L.Z. Zahradnikova, J. Tomandl, J. Pharm. Biomed. Anal. 52 (2010) 557.
[31] A.M. Idris, J. Pharmacol. Toxicol. Methods 56 (2007) 330.