Journal of Chromatography B, 891–892 (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Determination of a highly selective mixed-affinity sigma receptor ligand, in rat plasma by ultra performance liquid chromatography mass spectrometry and its application to a pharmacokinetic study

Seshulatha Jamalapuram[a], Pradeep K. Vuppala[a], Christophe Mesangeau[b], Christopher R. McCurdy[b,c], Bonnie A. Avery[a,*]

[a] Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[b] Department of Medicinal Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[c] Department of Pharmacology, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

ARTICLE INFO

Article history:
Received 8 July 2011
Accepted 12 December 2011
Available online 20 December 2011

Keywords:
Sigma receptor ligand
UPLC/MS
Method validation
Pharmacokinetics

ABSTRACT

A selective, rapid and sensitive ultra performance liquid chromatography mass spectrometry (UPLC/MS) method was developed and validated to quantitate a highly selective mixed-affinity sigma receptor ligand, CM156 (3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*] thiazole-2(3*H*)-thione), in rat plasma. CM156 and the internal standard (aripiprazole) were extracted from plasma samples by a single step liquid–liquid extraction using chloroform. The analysis was carried out on an ACQUITY UPLC™ BEH HILIC column (1.7 μm, 2.1 mm × 50 mm) with isocratic elution at flow rate of 0.2 mL/min using 10 mM ammonium formate in 0.1% formic acid and acetonitrile (10:90) as the mobile phase. The detection of the analyte was performed on a mass spectrometer operated in selected ion recording (SIR) mode with positive electrospray ionization (ESI). The validated analytical method resulted in a run time of 4 min and the retention times observed were 2.6 ± 0.1 and 2.1 ± 0.1 min for CM156 and the IS, respectively. The calibration curve exhibited excellent linearity over a concentration range of 5–4000 ng/mL with the lower limit of quantification of 5 ng/mL. The intra- and inter-day precision values were below 15% and accuracy ranged from −6.5% to 5.0%. The mean recovery of CM156 from plasma was 96.8%. The validated method was applied to a pilot intravenous pharmacokinetic study in rats.

1. Introduction

Cocaine abuse continues as a major problem in the United States due to its powerful psychological addictive properties [1]. The most common manifestations of drug toxicity are agitation, kindling (seizures and psychosis), neurotoxicity and stroke damage. Cocaine has been shown to block the reuptake of dopamine from the synapse by inhibiting the dopamine transporter and thus causing euphoria [2]. There has been a wide spread research effort to develop new molecular entities against the rewarding and adverse effects of cocaine by targeting dopamine, adrenergic and glutamate receptors. However, because of its innumerable action sites, currently no approved medication is available to treat cocaine toxicity [1]. Therefore new compounds that can mitigate the actions of cocaine are needed for the treatment of cocaine addiction [3]. Interactions of cocaine with sigma receptors were revealed by the blockade of locomotor stimulant effects, attenuation of convulsions and rewarding effects of cocaine by selective sigma receptor antagonists like BD 1008 [4–6]. Also, cocaine has been shown to bind to sigma receptors with an affinity of about 2 μM, suggesting that sigma receptors are likely to represent promising targets for the development of anti-cocaine agents [1,7–10].

Sigma receptors, discovered in 1976 by Martin and co-workers, were originally thought to be a class of opioid receptors; however numerous studies have proved them as unique receptors distinct from other proteins [1,7,10,11]. To date, two subtypes of sigma receptors have been identified; sigma-1 and sigma-2, based on the binding and drug discrimination studies [7,12]. The sigma-1 receptor exhibits high affinity and stereoselectivity for the (+)-isomers of benzomorphans, in contrast sigma-2 receptor prefers the (−)-stereoisomers. The sigma-1 receptor was cloned in 1996 [13,14]. However, the sigma-2 receptor has not yet been cloned, and the plausible reason may be lack of selective sigma-2 ligands [15,16]. Sigma receptors are widely distributed in the central nervous system (CNS) with the highest concentrations found in the substantia nigra, and the cerebellum. These regions are engaged

* Corresponding author. Tel.: +1 662 915 5163; fax: +1 662 915 1177.
E-mail address: bavery@olemiss.edu (B.A. Avery).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.12.013

in reward, addiction and motor control. In addition, sigma receptors are distributed in peripheral organs such as the heart, liver and gastrointestinal tract [1,2,17].

Several studies have shown that many antidepressant and antipsychotic drugs bind to sigma-1 receptors [12,16,18]. Thus selective sigma-1 receptor ligands have been proposed as potential candidates in the treatment of neuropsychological disorders such as psychotic major depression, Alzheimer's disease and Schizophrenia [19,20]. A major concern with the currently existing sigma receptor ligands is that most of them are not purely sigma selective. Other than sigma receptors, they bind to dopamine transporters or *N*-methyl-D-aspartate (NMDA) receptors. Thus studies with these non sigma-preferring ligands can further complicate the understanding of the role of sigma receptors [11]. Therefore, highly selective sigma ligands play a vital role in the development of therapeutic agents to treat cocaine abuse and various psychological disorders [11,16].

CM156 (3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione), a cyclohexylpiperazine derivative, is a highly selective sigma receptor antagonist shown to possess high affinity for sigma-1 and sigma-2 receptors in the nanomolar and subnanomolar range [7,15]. Pharmacological studies demonstrated that CM156 was the best sigma receptor ligand available to date with highest selectivity and preferential affinity to sigma receptors. CM156 was shown to significantly attenuate the expression of cocaine induced behavioral sensitization and place conditioning behaviors possibly by interfering with access of cocaine to sigma receptors [11]. This study suggests the involvement of sigma receptors in the subchronic effects of cocaine such as sensitization and the reward properties [17,21]. Cocaine also initiates multiple signal transduction pathways that modify the activities of neurotrasmitter systems such as glutamatergic, dopaminergic and cholinergic systems. These systems can be regulated by sigma receptors. The novel sigma receptor antagonist CM156, is anticipated to modulate and reduce the actions of these neurotransmitter systems activated by cocaine [11].

In order to better understand the pharmacokinetic characteristics of CM156 we developed and validated a rapid, sensitive and reliable UPLC/MS method for the quantitative determination of CM156 in plasma. A high sample throughput was achieved by simple sample preparation and short chromatographic run times under isocratic conditions using this method. The developed bioanalytical method was validated for specificity, linearity, precision, accuracy and lower limit of quantification. This method was ultimately used in a pilot study to assess the pharmacokinetic parameters of CM156 in Sprague-Dawley rats after a single intravenous administration of the compound. Using the data obtained from the pilot study, this method will be further used to investigate the ADME properties of CM156 in the future.

2. Experimental

2.1. Chemicals and reagents

CM156, 3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione (≥99% purity), was synthesized as previously reported by Mesangeau et al. [15]. The internal standard (IS), aripiprazole (99% purity), was purchased from Sigma Aldrich (St. Louis, MO, USA). Formic acid was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate was purchased from Sigma Aldrich (St. Louis, MO, USA). Rat plasma was purchased from Innovative Research (Peary Court Novi, MI, USA). All solvents used were HPLC grade.

2.2. Preparation of calibration standards and quality control samples

The stock solution of CM156 was prepared by dissolving an accurately weighed amount of the compound in water to obtain a final concentration of 1 mg/mL. Diluting the stock solution with acetonitrile made a series of working standard solutions, at concentrations ranging from 50 to 40,000 ng/mL. The IS stock solution (1 mg/mL) was prepared in methanol and from this stock solution, a working standard solution of IS (3 μg/mL) was prepared by diluting the stock solution with acetonitrile. All stock solutions were stored at −20 °C and used within one week of preparation.

Calibration standards were prepared freshly at concentrations of 5, 10, 50, 100, 500, 1000, 2000 and 4000 ng/mL by spiking blank rat plasma (100 μL) with the working standard solutions. According to US-FDA Bioanalytical Method Validation Guidance we have selected the three Quality control (QC) concentrations representing the entire range of the calibration curve [22]. The lower QC was selected within 3× the lower limit of quantification (LLOQ). The middle and upper QC's were selected to represent the middle and higher concentrations of the standard curve. QC samples were prepared in a similar way at concentrations of 10, 400 and 3000 ng/mL.

2.3. Sample preparation

A liquid–liquid extraction method was used to extract CM156 from all of the rat plasma samples including calibration standards, QC samples. Prior to extraction, the rat plasma samples (100 μL) were thawed at room temperature, spiked with 10 μL of IS and vortexed for 30 s. The mixture was extracted with chloroform. A volume of 800 μL of chloroform was added to each sample, vortexed (VWR Scientific Inc., Radnor, PA, USA) for 15 min and centrifuged at 10,000 × *g* for 10 min at 4 °C. A fixed aliquot (750 μL) of the organic phase was then transferred in to an eppendorf tube (Fisher Scientific, Pittsburgh, PA, USA) and dried in a vacuum oven (Precision Scientific, Winchester, VA, USA) at 25 °C. The resulting residue was reconstituted with 100 μL of acetonitrile and transferred into a micro sample insert (Microsolv Technology Corp., Eatontown, NJ, USA) that was pre-installed in a 1.5 mL autosampler vial for analysis.

2.4. Liquid chromatographic and mass spectrometric conditions

The chromatographic separations were performed on an Acquity UPLC (Waters Corp., Milford, MA, USA) equipped with a binary solvent manager, vacuum degasser, temperature controlled column compartment, and an autosampler. Chromatographic separations were performed on a Waters Acquity UPLC™ BEH HILIC column (1.7 μm, 2.1 mm × 50 mm) using a mobile phase of 10 mM ammonium formate containing 0.1% formic acid and acetonitrile (10:90, v/v). The flow rate was set at 0.2 mL/min and resulted in a total run time of 4 min. The injection volume was 10 μL and the column temperature was held constant at 25 °C. The mass spectrometric detection was carried out on a Micromass Quattro micro™ system (Waters Corp., Manchester, UK) using the positive ion mode. The following MS parameters were set for optimal detection of CM156 compound: a capillary voltage of 4.74 kV; a cone voltage of 36 V; an extractor voltage of 5 V; a RF lens voltage of 0.5 V; a source temperature of 60 °C and a desolvation temperature of 250 °C. The desolvation and cone gas flows were set at 500 and 72 L/h, respectively. Quantification was carried using selected ion recording (SIR) for CM156 *m*/*z* 390 and IS *m*/*z* 448, with a dwell time of 500 ms. Data acquisition and data processing were performed using Masslynx 4.1 software (Micromass, Manchester, UK) and Microsoft Excel 2007.

2.5. Method validation

Analytical method validation assays were performed as per the United States Food and Drug Administration (US-FDA) Bioanalytical Method Validation Guidance [22]. The validation of the UPLC/MS method included linearity, sensitivity, recovery, matrix effect, precision, accuracy, selectivity, and stability.

2.5.1. Linearity and sensitivity

An eight-point calibration curve 5, 10, 50, 100, 500, 1000, 2000 and 4000 ng/mL was constructed by plotting the ratio of the analyte peak area/IS peak area versus analyte concentration. The linearity of the calibration curve was evaluated by linear regression analysis. The sensitivity of the developed method was determined using LLOQ, the lowest concentration on calibration curve with a relative standard deviation (RSD) and relative error (RE) of less than 20%. The LLOQ was evaluated by analyzing samples in six replicates on three consecutive days [23]. The limit of detection (LOD) is defined as the analyte concentration that gives rise to peak whose height is 3 times that of baseline noise.

2.5.2. Selectivity

The selectivity of the developed method was investigated for the assessment of potential interferences of analyte and IS from endogenous substances. This was evaluated by comparing the chromatograms of six different lots of blank rat plasma (non pooled) containing sodium heparin, with the corresponding spiked plasma samples with CM156 and IS.

2.5.3. Recovery and matrix effect

The extraction recovery of CM156 from rat plasma was determined at concentrations of 10, 400 and 3000 ng/mL by comparing the peak area ratios of compound and IS. Recovery was calculated by comparing the plasma samples spiked with compound and IS before extraction with the plasma samples to which compound and IS were added after extraction.

The matrix effect, due to co-eluting plasma components, was evaluated by spiking six different lots of blank rat plasma with the QC solutions. The matrix effect of CM156 was determined at three QC levels (10, 400 and 3000 ng/mL) by comparing the peak area ratios of standards prepared in plasma with peak area ratios of standards prepared in acetonitrile.

2.5.4. Precision and accuracy

The precision and accuracy of the assay were determined by analyzing QC samples at three different concentrations (10, 400 and 3000 ng/mL). To evaluate intra-day accuracy and precision, QC samples were analyzed in six replicates at each concentration level. The inter-day accuracy and precision was determined by analysis of QC samples on three consecutive days. The concentrations were calculated based on calibration curve. The precision of the developed method was expressed as relative standard deviation (RSD) and accuracy as relative error (RE). The intra-day and inter-day precisions were required to be below 15%, and the accuracy to be within ±15%.

2.5.5. Stability

The stability of CM156 in rat plasma was determined by the analysis of six replicates of QC samples (10, 400 and 3000 ng/mL) exposed to various storage conditions. For freeze–thaw stability studies, unprocessed QC samples were subjected to three freeze–thaw cycles. Each sample was stored at −20 °C for 24 h and thawed at room temperature, after which the samples were refrozen for 12–24 h under the same conditions. At the end of each cycle, the samples were processed, analyzed and compared with the freshly prepared QC samples. For the short-term temperature stability study, unprocessed QC samples were kept at room temperature for 12 h, which exceeds the routine preparation time of the samples. At different time points, the samples were processed, analyzed and compared with the freshly prepared QC samples. The post operative stability during storage in the autosampler was assessed by re-injecting the samples that were held in the autosampler at 25 °C for 24 h. To determine long-term stability, QC samples were stored at −20 °C for 1 month which exceeds the time between sample collection and sample analysis.

2.6. Application to a pharmacokinetic study

The developed and validated UPLC/MS method was used to determine the pharmacokinetic parameters of CM156 in rats after the intravenous administration of the compound. The Institutional Animal Care and Use Committee (IACUC) of the University of Mississippi approved the animal experimental protocol. Six male Sprague-Dawley rats (180–200 g) were obtained from Harlan Laboratories (Indianapolis, IN, USA), which had already inserted polyethylene cannulas into the right jugular vein. The rats were housed in metabolic cages and allowed free movement and access to water during the whole experiment. The rats were fasted for 12 h before dosing and for the first 4 h after dosing. CM156 (8 mg) was dissolved in saline (2 mL) for intravenous administration. A single intravenous bolus of CM156 was injected at a dose of 5 mg/kg through the jugular vein cannula. The formulation was filtered through a 0.2 μm syringe filter prior to administration. The i.v. solution was administered via the jugular vein cannula, after which the cannula was flushed with 0.2 mL heparinized saline to ensure complete administration of the dose.

Blood samples were collected through the indwelling cannula into heparinized micro centrifuge tubes at 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480 min. An initial blood volume of 0.05 mL was withdrawn to clear the line of heparinized saline. A fresh syringe was then used to withdraw a 0.25 mL blood sample that was placed in a micro-centrifuge tube. After each blood sampling, 0.25 mL of heparinized saline solution was used to flush the catheter. Blood samples were immediately centrifuged at 10,000 × *g* for 20 min at 4 °C using an accuspin Micro 17R centrifuge (Fisher Scientific, Pittsburgh, PA, USA). Plasma was separated from all blood samples and transferred in to 1 mL micro centrifuge tubes and were frozen at −20 °C until analysis. The pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin 5.2 (Pharsight, Mountain View, CA, USA).

3. Results and discussion

3.1. Chromatography

Because CM156 is a polar compound, it did not retain well on a traditional C_{18} column. Consequently, based on the retention time of the compound and the separation efficiency of the column, an Acquity BEH HILIC column was selected to develop the UPLC assay. Aripiprazole was chosen as the IS due to its similar chromatographic characteristics, strong mass response in positive ESI mode and lack of endogenous interferences at *m*/*z* 448. Different concentrations of formic acid (0.05%, 0.1% and 0.2%) in aqueous phase were tested to improve the chromatographic peak shapes and increase the MS response. The results indicated that a solution of 0.1% of formic acid improved the peak shapes and MS response of CM156 and the IS, but was unable to produce baseline separation of the two compounds. To improve the separation, various amounts of

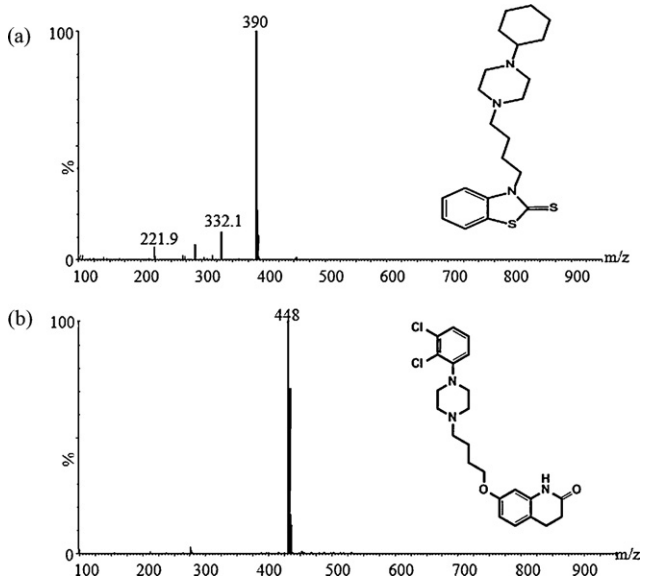

Fig. 1. Chemical structure and full mass scan of CM156 (a) and aripiprazole (b).

ammonium formate (1, 5 and 10 mM) were added to the aqueous phase. We determined that 10 mM ammonium formate produced the best separation of the two compounds. Acetonitrile was chosen as the organic phase because it produced a higher analyte response and led to lower background noise compared to methanol. The selected mobile phase consisted of 10 mM ammonium formate buffer solution containing 0.1% formic acid and acetonitrile (10:90 v/v), which was pumped at a flow rate of 0.2 mL/min. This resulted in retention times for CM156 and IS of 2.6 and 2.1 min, and a run time of 4 min. As the protein precipitation alone did not result in complete purification of the plasma samples, a single-step liquid–liquid extraction was adopted in the present study to achieve high recovery with no interferences in minimum time. Chloroform was selected as the extraction solvent because it showed invariable recoveries ranging from 86.3 to 105.9% in the concentration range from 5 to 4000 ng/mL.

3.2. Mass spectrometry

ESI positive ion monitoring mode was chosen for the measurement of CM156 in rat plasma samples. Because CM156 was a basic compound, it captured the protons easily and gave maximum detector intensity in the positive ionization mode. Upon the direct injection of CM156 and the IS in to the mass spectrometer, singly protonated ions were found to be the most sensitive ions $[M+H]^+$. The compound exhibited better sensitivity in SIR mode than the multiple reaction mode. This is probably caused by insufficient fragmentation in the collision cell. Therefore, we selected SIR mode with the molecular ions at m/z 390 and m/z 448 as the target ions for the detection of CM156 and IS respectively. Full mass scans of CM156 and IS are shown in Fig. 1.

3.3. Method validation

3.3.1. Linearity of calibration curve and lower limit of quantification

Calibration standards of CM156 at concentration levels of 5, 10, 50, 100, 500, 1000, 2000 and 4000 ng/mL were extracted and assayed. A typical regression equation for the calibration curve was:

$$y = 0.047x + 0.1997$$

where y represents the peak area ratios of CM156 to the IS and x represents plasma concentrations of analyte. The RSD of slope was 4.7 and the RSD of the intercept was 6.6 ($n = 6$). The calibration curve was found to be linear over the concentration range from 5 to 4000 ng/mL resulting in a correlation coefficient $r^2 > 0.995$. The lower limit of quantification for CM156 in plasma was 5 ng/mL with precision (RSD) below 20% and accuracy (RE) within ±20%. This quantification was found to be sensitive enough to investigate the pharmacokinetic behavior of CM156 in preclinical studies. The LOD was estimated at 2 ng/mL.

3.3.2. Selectivity

The selectivity of the assay was assessed by comparing the chromatograms of six different lots of blank rat plasma with the corresponding spiked plasma. The representative chromatograms of blank plasma, plasma spiked with CM156 (400 ng/mL) and IS (300 ng/mL) are presented in Fig. 2. No interference were observed at the retention times of the CM156 (2.6 ± 0.1 min) or the IS (2.1 ± 0.1 min).

3.3.3. Recovery and matrix effect

The extraction recoveries of CM156 from rat plasma at the concentrations of 10, 400 and 3000 ng/mL were 97.2 ± 5.6%, 100.4 ± 6.9% and 101.5 ± 7.8%, respectively. The extraction recovery of IS from rat plasma was 58.2% at a concentration of 300 ng/mL. Recovery of the internal standard was consistent and reproducible. The matrix effects of CM156 were between 90% and 105%. The matrix effect of IS was 98.5%. These results indicated that the influence of co-eluting substances on the ionization of the analyte and IS was negligible.

3.3.4. Precision and accuracy of the assay

The accuracy and the intra and inter-day precision of the analytical method were evaluated with six replicates at three different concentrations 10, 400 and 3000 ng/mL. The intra-day precision ranged from 3.5% to 4.4% and the inter-day precision ranged from 2.6% to 5.6%. The accuracy of the assay ranged from −6.5 to 5.0%. The data obtained was within the acceptable limits and the method was precise and accurate. Table 1 summarizes intra- and inter-day precision and accuracy.

3.3.5. Stability

The stability of CM156 was evaluated under different storage conditions at three concentrations (10, 400 and 3000 ng/mL). The results indicated that CM156 was stable in rat plasma stored at room temperature for 12 h, at −20 °C for 1 month and during three

Table 1
Precision and accuracy data for CM156 in rat plasma.

Spiked concentration (ng/mL)	Intra-day precision and accuracy ($n = 3$)			Inter-day precision and accuracy ($n = 3$)		
	Measured concentration (mean ± SD, ng/mL)	RSD (%)	RE (%)	Measured concentration (mean ± SD, ng/mL)	RSD (%)	RE (%)
10	10.5 ± 0.4	3.8	5.0	9.4 ± 0.5	5.6	−6.5
400	411.0 ± 14.5	3.5	2.8	404.3 ± 10.7	2.6	1.1
3000	2932.4 ± 130.2	4.4	−2.3	2993.3 ± 88.7	2.9	−0.2

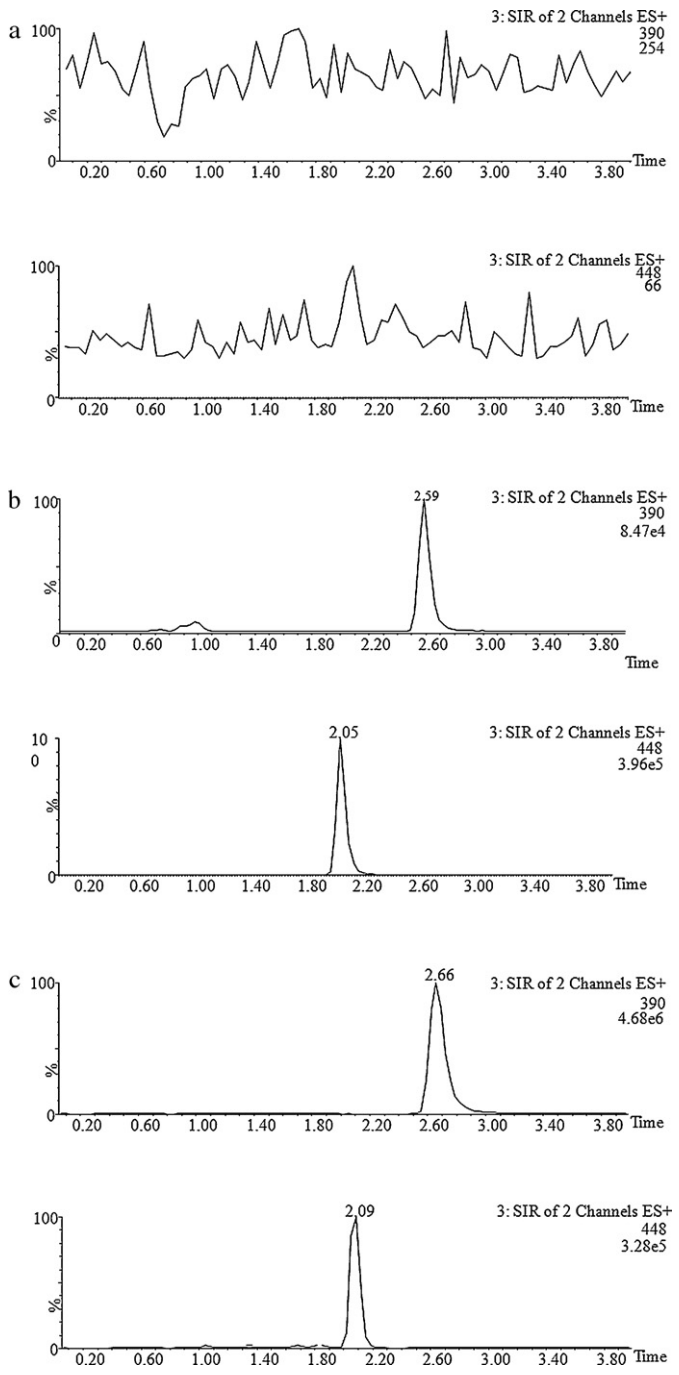

Fig. 2. Representative SIR chromatograms for CM156 and aripiprazole (IS) in rat plasma: (a) a blank plasma sample; (b) a blank plasma sample spiked with CM156 at LLOQ and aripiprazole (IS) at 300.0 ng/mL; (c) a rat plasma sample obtained at 1.0 h after a single intravenous injection at a dose of 5 mg/kg.

freeze/thaw cycles. The compound was also found to be stable in reconstituted samples when stored for 24 h in the autosampler at 25 °C. The stock solutions of CM156 and IS were stable for at least 6 h at room temperature and for one week at −20 °C. Results of the stability studies are shown in Table 2.

3.4. Application to a pharmacokinetic study in rats

The validated method described above was successfully applied to a pharmacokinetic study of CM156 in six male Sprague-Dawley rats. The method was found to be sensitive enough to quantify the plasma concentration 8 h after i.v. dosing. The mean plasma concentration–time profile is shown in Fig. 3. The pharmacokinetic parameters are presented in Table 3. After administration of a single i.v. dose of 5 mg/kg, the C_{max} of CM156 was 1.3 ± 0.2 μg/mL. The plasma concentrations declined very quickly indicating a rapid distribution of the novel sigma receptor ligand in to the tissues. The distribution of CM156 was found to be extensive (9.6 L/kg), which may be a desirable property for a compound acting on central nervous system. The elimination of CM156 from the systemic circulation was rapid as evidenced by its high clearance (6.2 L/h/kg) and relatively short half-life (60 min).

Table 2
Results of the stability studies of CM156 at different storage conditions.

Storage condition	Concentration (ng/mL)		RSD (%)	RE (%)
	Spiked	Measured		
Three freeze thaw cycles	10	9.2 ± 1.1	12.3	−6.6
	400	406.4 ± 8.8	2.2	1.6
	3000	2879.4 ± 209.8	7.3	−4.0
Long term for 30 days (−20 °C)	10	11.2 ± 0.9	8.6	12.8
	400	394.8 ± 17.8	4.5	−1.3
	3000	2879.4 ± 243.1	7.8	3.9
Short term for 12 h (25 °C)	10	8.6 ± 0.7	8.3	−12.3
	400	403.6 ± 9.2	2.2	2.3
	3000	2879.4 ± 145.2	5.0	4.0
Auto sampler for 24 h (25 °C)	10	10.0 ± 0.2	3.0	2.2
	400	404.4 ± 4.7	1.2	1.1
	3000	2901.1 ± 59.2	2.0	−3.3

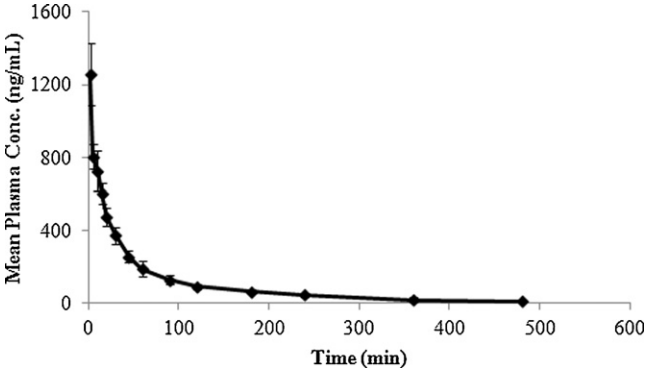

Fig. 3. Mean plasma concentrations of CM156 after a single intravenous injection of to rats at a dose of 5 mg/kg (n = 6).

Table 3
Pharmacokinetic parameters of CM156 in rats following a single intravenous dose of 5 mg/kg (n = 6).

Parameter	Mean ± SD
$t_{1/2}$ (h)	1.1 ± 0.4
C_{max} (μg/mL)	1.3 ± 0.2
$AUC_{0\rightarrow\infty}$ (μg min/mL)	48.1 ± 6.3
V_d (L/kg)	9.6 ± 1.4
CL (L/h/kg)	6.2 ± 1.0
MRT (min)	90.1 ± 13.0

Elimination half-life ($t_{1/2}$), peak plasma concentration (C_{max}), area under the plasma concentration–time curve (AUC), volume of distribution (V_d), total clearance (CL), mean residence time (MRT), and standard error (SE).

4. Conclusions

In this study, a rapid, simple and sensitive UPLC/MS method was developed and validated for the determination of CM156 in rat plasma. This method showed excellent sensitivity, linearity, precision and accuracy. Furthermore, the validated method was

successfully applied to an intravenous pharmacokinetic study of CM156 in rats.

Acknowledgment

This work was supported in part by a grant from the National Institute on Drug Abuse (DA023205, CRM).

References

[1] A. Daniels, E. Ayala, W. Chen, A. Coop, R.R. Matsumoto, Eur. J. Pharmacol. 542 (2006) 61.
[2] T. Maurice, R.M. Fardon, P. Romieu, R.R. Matsumoto, Neurosci. Biobehav. Rev. 26 (2002) 499.
[3] S. Narayanan, C. Mesangeau, J.H. Poupaert, C.R. McCurdy, Curr. Top. Med. Chem. 11 (2011) 1128.
[4] M.C. Ritz, F.R. George, Psychopharmacology (Berlin) 129 (1997) 311.
[5] T. Hiranita, P.L. Soto, G. Tanda, J.L. Katz, J. Pharmacol. Exp. Ther. 332 (2010) 515.
[6] L.G. Ramirez, J.L. Green, T. Hiranita, T.A. Kopajtic, M. Mereu, A.M. Thomas, C. Mesangeau, S. Narayanan, C.R. McCurdy, J.L. Katz, G. Tanda, Biol. Psychiatry 69 (2011) 208.
[7] T. Maurice, T.P. Su, Pharmacol. Ther. 124 (2009) 195.
[8] R.R. Matsumoto, K.A. McCracken, B. Pouw, J. Miller, W.D. Bowen, W. Williams, B.R. De Costa, Eur J. Pharmacol. 411 (2001) 261.
[9] R.R. Matsumoto, K.L. Hewett, B. Pouw, W.D. Bowen, S.M. Husbands, J.J. Cao, A. Hauck Newman, Neuropharmacology 41 (2001) 878.
[10] S. Izenwasser, A.H. Newman, J.L. Katz, Eur. J. Pharmacol. 243 (1993) 201.
[11] Y.T. Xu, N. Kaushal, J. Shaikh, L.L. Wilson, C. Mesangeau, C.R. McCurdy, R.R. Matsumoto, J. Pharmacol. Exp. Ther. 333 (2010) 491.
[12] T. Hayashi, T.P. Su, CNS Drugs 18 (2004) 269.
[13] M. Hanner, F.F. Moebius, A. Flandorfer, H.G. Knaus, J. Striessnig, E. Kempner, H. Glossmann, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 8072.
[14] I.T. Lee, S. Chen, J.A. Schetz, Eur. J. Pharmacol. 578 (2008) 123.
[15] C. Mesangeau, S. Narayanan, A.M. Green, J. Shaikh, N. Kaushal, E. Viard, Y.T. Xu, J.A. Fishback, J.H. Poupaert, R.R. Matsumoto, C.R. McCurdy, J. Med. Chem. 51 (2008) 1482.
[16] J.A. Fishback, M.J. Robson, Y.T. Xu, R.R. Matsumoto, Pharmacol. Ther. 127 (2010) 271.
[17] R.R. Matsumoto, K.A. McCracken, B. Pouw, Y. Zhang, W.D. Bowen, Neuropharmacology 42 (2002) 1043.
[18] Y. Itazhak, Mol. Pharmacol. 36 (1989) 512.
[19] S.M. Stahl, CNS Spectr. 10 (2005) 319.
[20] T. Furuse, K. Hashimoto, Ann. Gen. Psychiatry 9 (2010).
[21] P. Romieu, V.L. Phan, R.M. Fardon, T. Maurice, Neuropsychopharmacology 26 (2002) 444.
[22] Food and Drug Administration, Center for Drug Evaluation and Research, May, 2001. Available from: http://www.fda.gov/cder/guidance.
[23] X. Liu, H. Zheng, W. Tang, Z. Qian, Y. Zhu, J. Wang, S. Yuan, X. Wen, C. Cao, H. Chen, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 25 (2011) 2554.