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Quantitative determination of MCC-555, a novel insulin sensitizer in beagle dog plasma by high-performance liquid chromatography with fluorescence detection

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Abstract

A sensitive high-performance liquid chromatographic method with fluorescence detection has been developed for determination of MCC-555 (5-[[6-(2-fluorbenzyl)-oxy-2-naphy] methyl]-2,4-thiazolidinedione) in beagle dog plasma. Sample preparation was done by protein precipitation with acetonitrile and a synthetic intermediate of MCC-555 (5-[[6-(2-fluorbenzyl)-oxy-2-naphy] methylene]-2,4-thiazolidinedione) was used as the internal standard (IS). The isocratic mobile phase consisted of acetonitrile–10 mmol/l sodium phosphate buffer (pH 4.5) (65:35, v/v) was delivered at a flow rate of 1 ml/min to a Kromasil C_{18} reversed-phase column (250 mm × 4.6 mm, 5 μm). The compounds were detected by fluorescence detection, using an excitation wavelength of 232 nm, and emission wavelength of 352 nm. Calibration curves of MCC-555 were linear in the concentration range of 0.005–2.0 μg/ml. Intra- and inter-day precision ranged from 3.4 to 5.4% and 3.0 to 8.8%, respectively. No endogenous interferences were observed with either MCC-555 or IS. The assay is simple, economical, precise, and is directly applicable to pharmacokinetic studies in beagle dogs involving three dose administrations.

Keywords: MCC-555; High-performance liquid chromatography; Pharmacokinetics

1. Introduction

Thiazolidinediones (TZDS) are anti-diabetic agents that enhance insulin sensitivity through activating peroxisome proliferator-activated receptor (PPAR) γ [1]. TZDS including troglitazone [2], rosiglitazone [3] and pioglitazone [4] comprise a new class of oral antidiabetic agents, which reduce plasma glucose concentrations in Type 2 diabetes mellitus models due to enhancement of insulin sensitivity, whereas these TZDS are not potent to activate PPAR α and PPAR δ [5].

MCC-555 (Fig. 1), is a novel TZD which has unique characteristics of ability to activate not only PPAR γ but also PPAR α and δ on vascular cell adhesion molecule-1(VCAM-1) expression in vascular endothelial cell (ECs) [6]. This effect is exerted probably through activation of PPAR α and/or PPAR δ, rather than PPAR γ, mediating down-regulation of nuclear factor-κB activity [7]. It has the potentials to improve metabolic status and insulin sensitivity in obese Zucker and Zucker Diabetic Fatty (ZDF) rats [8], and attenuate the development of overt diabetes in young ZDF rats [9]. It has greater anti-hyperglycemic potency than that of rosiglitazone, and pioglitazone in KK-A^y mice yet has lower affinity for PPAR γ than rosiglitazone [10]. Moreover, the effect of MCC-555 on PPAR γ transcriptional activity is highly context-specific such that it can function as a full agonist, partial agonist or antagonist that depends on the cell type or DNA binding site [10].

Several methods have been reported for the extraction and detection of thiazolidinediones anti-diabetic drugs in biological fluids, such as high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [11], fluorescence detection [12] and mass spectrometry [13] and micellar elec-

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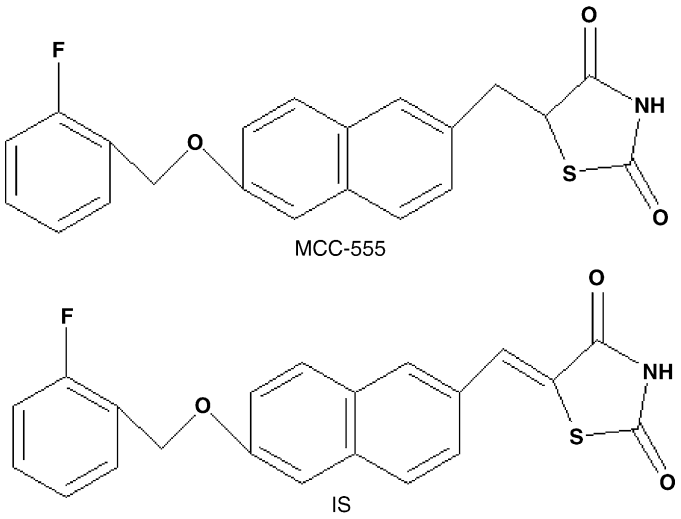

Fig. 1. Structures of MCC-555 and the internal standard (IS).

trokinetic capillary chromatography (MEKC) with diode-array detection (DAD) [14].

The preclinical pharmacokinetic data of MCC-555 is to be generated, which requires the development of an analytical method for MCC-555 in biological matrices. To our knowledge, there is still no method described for the determination of MCC-555 in biological fluids. In this paper, we established an HPLC fluorescence method with simple sample processing to quantify MCC-555 in beagle dog plasma for the first time. The method exhibits good reproducibility and high sensitivity, specificity and robustness when used to determine MCC-555 plasma concentrations after three doses (2.5, 5.0, 10.0 mg/kg) to beagle dogs.

2. Experimental

2.1. Materials and reagents

MCC-555 and IS (Fig. 1, purity of both ≥99.7%) were provided by Shanghai Jiahua Medicine Science, Co. Ltd. (Shanghai, China). Acetonitrile of HPLC grade was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade. Redistilled water was used for the preparation of all solutions and 0.45 μm pore size filters (Millipore, MA) was used to filter the solutions.

2.2. Chromatographic system and conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AD VP pump coupled with a SPD-10A fluorescence detector, SIL-10AD VP automated sample injector, thermostatted column compartment CTO-10AS VP and N2000 workstation (Zhejiang University, Hangzhou, China).

Separation was achieved with a Kromasil C_{18} reversed-phase column (5 μm, 250 mm × 4.6 mm i.d.), protected by a Diamonsil™ ODS guard column, and an isocratic mobile phase of acetonitrile–10 mmol/l sodium phosphate buffer (pH 4.5) (65:35, v/v) delivered at a flow rate of 1 ml/min. MCC-555 and IS were monitored at λ_{ex} of 232 nm, λ_{em} of 352 nm. The column temperature was 35 °C and the injection volume was 20 μl. The total run time was less than 15 min.

2.3. Preparation of standard and quality control solutions

The stock solution of MCC-555 (1.0 mg/ml) was prepared in methanol. Working standard solutions of MCC-555 were obtained in the concentrations (0.1, 1.0, 10.0, 100 μg/ml) by further dilutions of the stock solution with methanol. The internal standard (IS) was prepared at a concentration of 1 μg/ml in methanol. The working standard solutions of MCC-555 were used to prepare calibration standards and quality control (QC) samples. All the solutions were stored at 4 °C and brought to room temperature before use.

2.4. Sample preparation

To a 100 μl aliquot of dog plasma, 300 μl acetonitrile solution containing 1.0 μg/ml of the internal standard was added and the mixture was vortexed for 30 s. After vortex-mixing, the samples were centrifuged at 12000 rpm for 10 min, the supernatant was transferred to microcentrifuge Eppendorf tube and 20 μl was injected directly into the chromatographic system.

2.5. Validation of the analytical method

The methods were validated according to International Conference on Harmonization Q2B guidelines [15] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

2.5.1. Linear range and limit of quantification

Calibration curves consisted of nine standard concentrations of MCC-555 spiked in beagle dog plasma: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 μg/ml. The extraction procedure and HPLC analysis as described above were performed on the calibration samples. The calibration curve was constructed by plotting the peak area ratios of MCC-555 to IS against nominal MCC-555 concentrations and generated on five consecutive days. The limit of quantification (LOQ) was defined as the lowest concentration of MCC-555 giving a signal to noise of 10:1 with a precision less than 15% [15].

2.5.2. Precision and accuracy

Validation samples were prepared and analyzed to evaluate the intra-day and inter-day precision and accuracy, which were determined by quantitating five replicates at concentrations of 0.04, 0.4 and 1.4 μg/ml on the same day and five consecutive days. Mean, standard deviation, and relative standard deviation (R.S.D.) were calculated from QC values and used in the estimation of intra- and inter-day precision. Accuracy was assessed by comparison of the calculated mean concentrations to nominal concentrations.

2.5.3. Stability

The freeze-thaw stability was evaluated by analyzing the QC samples at the concentrations of 0.04, 0.4 and 1.4 μg/ml

after undergoing three freeze (−20 °C)-thaw (ambient) cycles. The stability of MCC-555 in beagle dog plasma was assessed by placing the QC samples at room temperature for up to 6 h before being extracted and analyzed. The stability of MCC-555 in the reconstituted methanol solutions was determined by keeping the reconstituted methanol solutions of the QC samples at room temperature for up to 12 h before analysis.

2.5.4. Extraction recovery

The recoveries of MCC-555 were determined by spiked samples at three concentrations: 0.04, 0.4 and 1.4 μg/ml. The extraction recoveries were calculated by comparing peak areas from spiked samples to the same amounts of unextracted MCC-555 solutions. The internal standard at the concentration of 1 μg/ml was determined in the same way.

2.6. Pharmacokinetic study

The pharmacokinetic study was carried out in three male and three female beagle dogs. The animals were used after being raised for one week. Before the day of administration, six beagle dogs were fasted overnight but were allowed access to water throughout the experimental period. Animals were given food 3 h after drug administration. MCC-555 was administered by gastric gavage at three doses (2.5, 5.0 and 10.0 mg/kg) as a 0.5% carboxymethyl-cellulose suspension, and three dose experiments were separated with a washout period of two weeks. Blood samples were collected in dried heparinized tubes before administration and post-dose at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h. The plasma was separated out by centrifugation at 3500 rpm for 10 min and was stored at −20 °C until analysis.

3. Results and discussion

3.1. Method development

3.1.1. Fluorescence spectra

As shown in Fig. 2, the fluorescence spectra of MCC-555 in the mobile phase (acetonitrile–10 mmol/l sodium phosphate buffer (pH 4.5) (65:35, v/v)) showed fluorescence detection at an excitation wavelength of 232 nm and emission wavelength of 352 nm.

3.1.2. Mobile phase

The composition of mobile phase was optimized through several trials to achieve good resolution and symmetric peak shapes of analytes as well as short run time. It was found that acetonitrile–10 mmol/l sodium phosphate buffer (pH 4.5) (65:35, v/v) could achieve our purpose and was finally adopted as the mobile phase.

3.1.3. Choice of internal standard

The selection of the internal standard was an arduous and hard process, we have tried Galanthamine, Naproxen, Rosiglitazone and Metoprolol, but all failed partly for the short retention time or being interfered by endogenous matrix. A synthetic intermediate of MCC-555, 5-[[6-(2-fluorbenzyl)-oxy-2-naphy]methylene]-2,4-thiazolidinedione, was finally selected as the internal standard, because it was also a fluorescent compound and had a suitable retention time and a good resolution from MCC-555 under the chromatographic conditions in this study. There was no endogenous interferences in the regions where MCC-555 and the IS eluted.

3.2. Validation

3.2.1. Specificity

The resulting chromatograms were essentially free from endogenous interferences (Fig. 3), which included a blank plasma sample, a plasma sample spiked with MCC-555 0.2 μg/ml and a plasma sample obtained 3 h after oral dose of MCC-555 5.0 mg/kg. Retention times were approximately 9.7 and 10.6 min for IS and MCC-555, respectively, and the peak shape and the resolution between MCC-555 and IS were satisfactory and suitable.

3.2.2. Linearity, precision and accuracy

Peak area ratios of MCC-555 to IS were measured. An excellent linear relationship was demonstrated between peak area ratio of MCC-555 to IS and the corresponding plasma concentrations of MCC-555 over the range of 0.005–2.0 μg/ml. The linear regression equation was $y = 2.2035x - 0.0005$ ($r^2 = 0.9999$). The

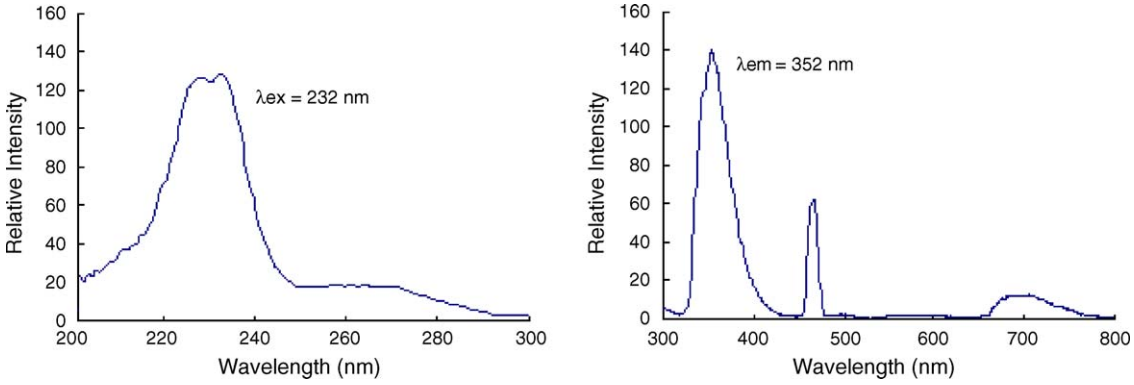

Fig. 2. The fluorescence spectra of MCC-555 in the mobile phase using an excitation wavelength of 232 nm and emission wavelength of 352 nm.

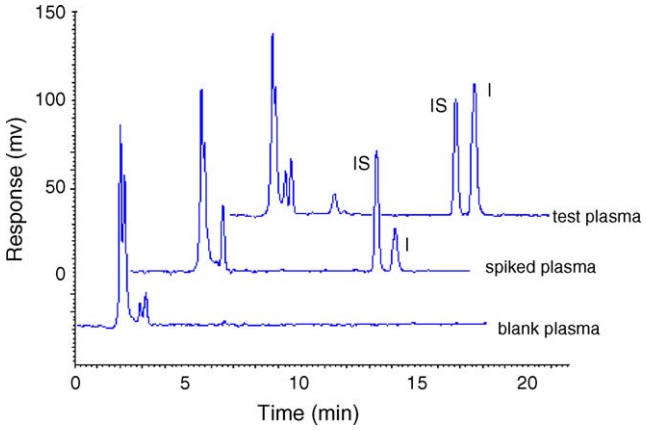

Fig. 3. Representative Chromatograms of extracted blank plasma, plasma spiked with 0.2 μg/ml of MCC-555 (I) and 1 μg/ml of the IS, and test plasma at 3 h after the dose of 5 mg/kg (concentration 1.305 μg/ml).

limits of quantification (LOQ) of MCC-555 in plasma were 0.005 μg/ml.

Table 1 showed a summary of intra- and inter-day precision and accuracy of QC samples. Intra- and inter-day R.S.D. ranged from 3.0 to 8.8% and accuracy ranged from 97.5 to 113.7%. The results of intra-day and inter-day analysis indicated that the method was accurate, reliable and reproducible.

3.2.3. Analyte stability and extraction recovery

MCC-555 was found to be stable after three freeze-thaw cycles in plasma. The recoveries of the QC samples were ranged from 94 to 113%. The stability of the analyte in the plasma and the reconstituted methanol at room temperature has also been investigated. MCC-555 was proved to be stable in the dog plasma for at least 6 h (R.S.D. < 7.8%), and stable in the reconstituted methanol at room temperature for more than 12 h (R.S.D. < 8.4%). Table 2 listed the mean extraction recoveries and the coefficients of variation (R.S.D. %) for MCC-555 at each concentration, and the recoveries ranged from 81.2 to 90.9%. So, it is expected that the present method would be applicable to the pharmacokinetic study of MCC-555 in beagle dogs.

Table 1
Results of precision and accuracy for MCC-555 in plasma ($n = 5$)

Concentration (μg/ml)		Precision (R.S.D., %)	Accuracy (%)
Spiked	Observed (mean ± S.D.)		
Intra-assay precision			
QC samples			
0.04	0.040 ± 0.001	3.4	99.0
0.4	0.390 ± 0.021	5.4	97.5
1.4	1.434 ± 0.060	4.2	102.4
Inter-assay precision			
QC samples			
0.04	0.044 ± 0.004	8.8	109.5
0.4	0.455 ± 0.031	6.9	113.7
1.4	1.424 ± 0.043	3.0	107.7

Table 2
Extraction recoveries of MCC-555 and IS ($n = 3$)

Drug	Concentration (μg/ml)	Recovery (%) (mean ± S.D.)	R.S.D. (%)
MCC-555	0.04	81.2 ± 7.8	9.7
	0.4	88.1 ± 3.9	4.5
IS	1.4	90.9 ± 5.8	6.4
	1.0	92.0 ± 4.8	5.2

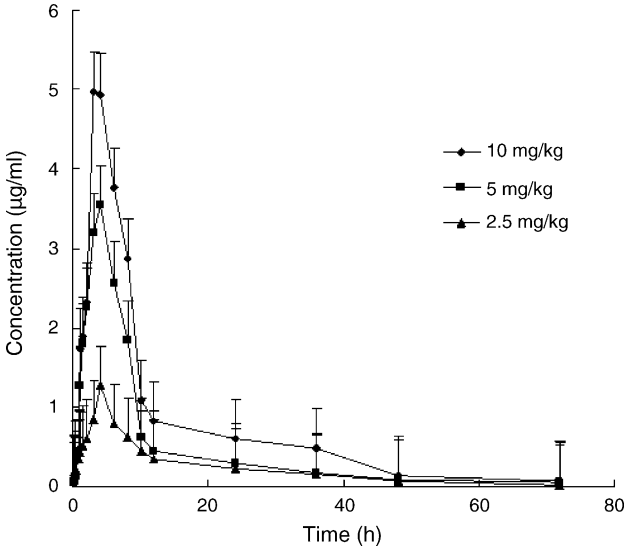

Fig. 4. Mean plasma concentration-time profiles of MCC-555 after three doses to six beagle dogs (2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg).

Table 3
Pharmacokinetic parameters obtained after three doses administrations of MCC-555 in beagle dogs (mean value of the six beagle dogs)

Dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)	$AUC_{0\sim72}$ (μg h/ml)	$AUC_{0\sim\infty}$ (μg h/ml))	MRT (h)
2.5	12.5	3.8	1.39	16.06	16.44	19.1
5.0	14.5	3.8	3.88	33.75	37.93	15.7
10.0	16.4	3.7	6.46	54.29	55.91	16.2

3.3. Application to pharmacokinetic study in beagle dogs

The method described here was successfully applied to study the pharmacokinetics of MCC-555 in beagle dogs. The mean plasma concentration-time curves in rats after oral administration of MCC-555 at three doses were illustrated in Fig. 4. Pharmacokinetic parameters of the three dose groups (2.5, 5, 10 mg/kg i.g.) were listed in Table 3. Area under the plasma concentration versus time curve (AUC) increased with the dose, showing apparent dose-dependent relationship ($r = 0.942$).

4. Conclusion

In this paper a HPLC method for the determination of MCC-555 in beagle dog plasma has been developed. The method allowed for simple and economical detection of MCC-555 in small volumes of beagle dog plasma, using protein precipitation

coupled with sensitive fluorescent detection using an excitation wavelength of 232 nm and emission wavelength of 352 nm. The chromatographic assay showed good characteristics of specificity, sensitivity and precision, allowing for numerous samples to be processed in pharmacokinetic studies of MCC-555.

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