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Determination of TA-0201, a novel orally active non-peptide endothelin antagonist, in rat plasma and tissues by a liquid chromatography–electrospray ionization–tandem mass spectrometry system

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Abstract

N-[6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-5-(4-methylphenyl)-4-pyrimidinyl]-4-(2-hydroxy-1, 1-dimethylethyl) benzenesulfonamide sodium salt (TA-0201) is a novel orally active non-peptide antagonist for endothelin (ET) receptors. A sensitive and simultaneous determination method of TA-0201 and its major metabolites by liquid chromatography–tandem mass spectrometry (LC–MS/MS) in a selected reaction monitoring mode using [$^2H_6$]TA-0201 as the internal standard was developed, and the plasma and tissue concentrations of TA-0201 and its major metabolites were determined in a pharmacokinetic study. The lower limit of determination of plasma TA-0201 concentrations by this method was 0.1 ng/0.5 ml, and the between- and within-run accuracy and precision were both less than 5.1% in the calibration curve range of 0.1–50 ng/0.5 ml. This method was applied to determine the concentrations of TA-0201 and its metabolites in plasma and various target tissues, i.e. the heart, lung and kidney, after oral administration of TA-0201 (0.1 mg kg^{-1}) to male rats. TA-0201 and its major metabolite of a carboxylic acid form were detected in plasma and all the tissues 24 h after administration, their tissue concentrations being higher than those in plasma and still detectable at 72 h. Thus, this method could successfully be applied to study pharmacokinetic properties of TA-0201 in rats.

Keywords: Liquid chromatography–tandem mass spectrometry; Electrospray; Collision-induced dissociation; Selected reaction monitoring; Endothelin receptor antagonist; TA-0201; Plasma and tissue concentrations

1. Introduction

N-[6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-5-(4-methylphenyl)-4-pyrimidinyl]-4-(2-hydroxy-1, 1-dimethylethyl)benzenesulfonamide sodium salt (TA-0201) is a novel non-peptide antagonist against endothelin (ET) and under development by Tanabe Seiyaku Co., Ltd. (Fig. 1) [1,2]. In vitro, TA-0201 showed potent competitive inhibition of the ET receptor binding; it antagonized

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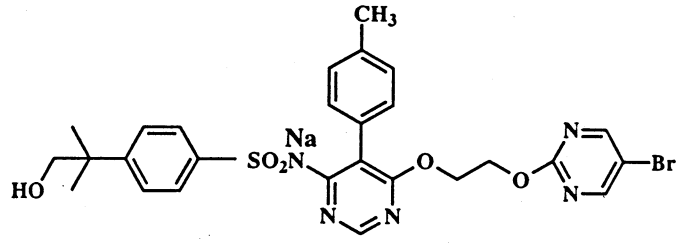
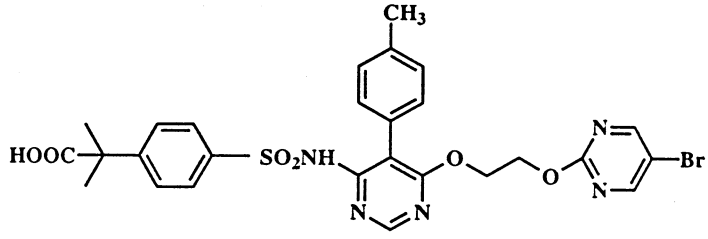
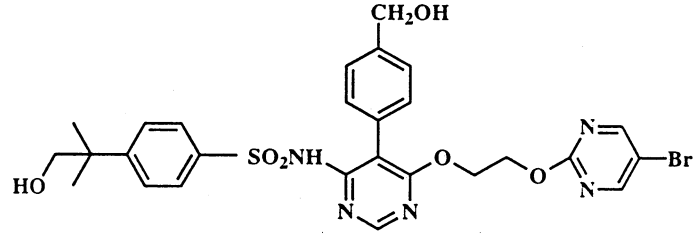


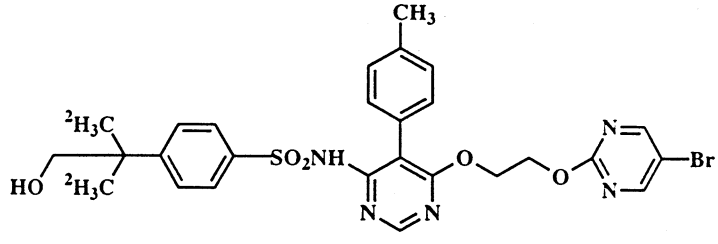
TA - 0201



Carboxylic acid metabolite (CA)



Diol metabolite (Diol)



I.S. ([$^2H_6$]-TA - 0201)

Fig. 1. Chemical structures of TA-0201, CA, Diol and I.S. ([$^2H_6$]TA-0201).

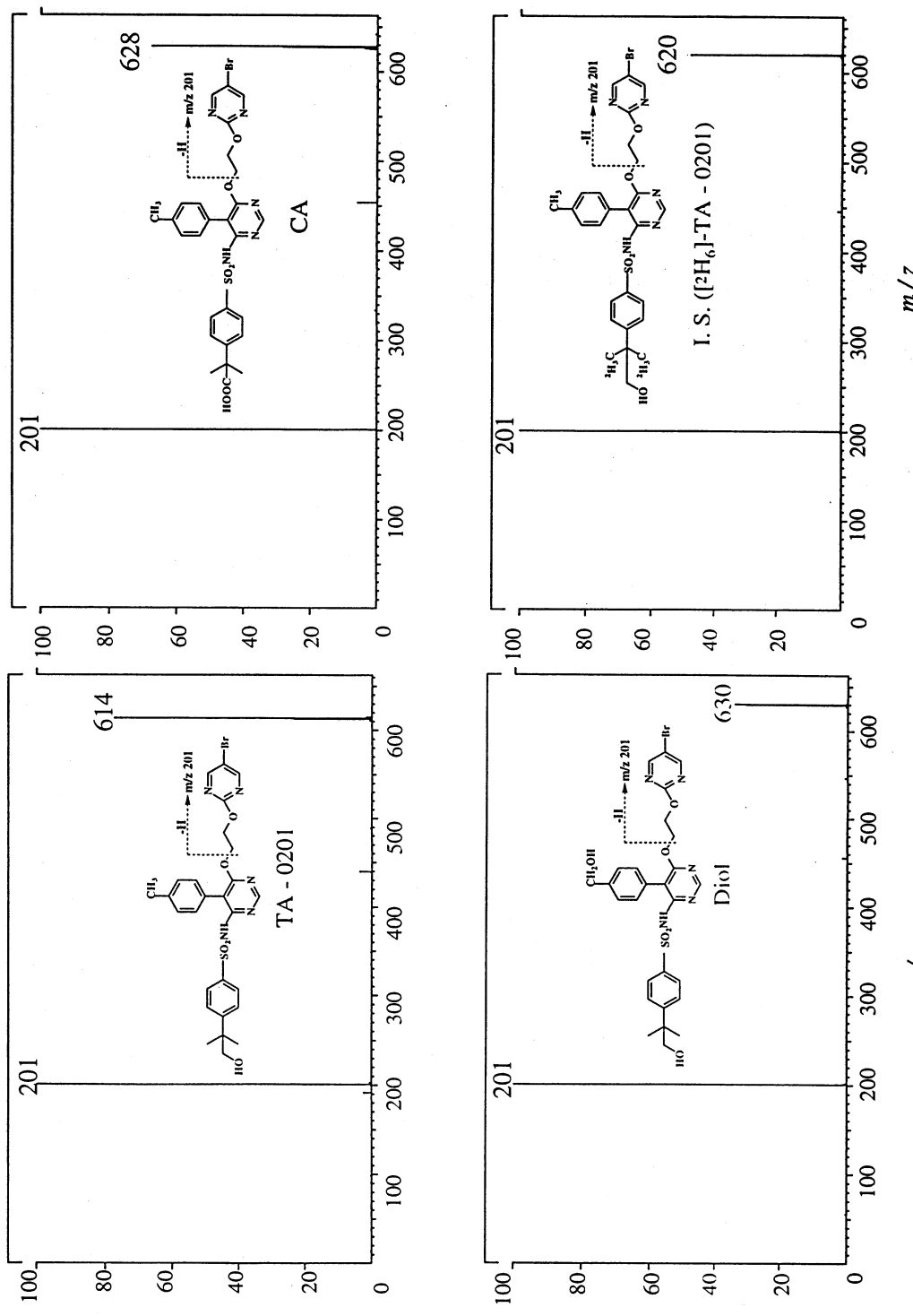


Fig. 2. Product ion mass spectra of TA-0201 (A), CA (B), Diol (C) and I.S. (D).

the specific binding of [^{125}I]ET-1 to cloned human receptors of ET_A and ET_B [3,4] with K_i values of 15 pM and 41 nM, respectively [5]. TA-0201 (0.01–10 mg kg^{-1}, iv) inhibited the pressor response to the exogenous big ET-1 (1 nmol kg^{-1}, iv) in anesthetized rats and dogs [5]. To support this effect of TA-0201 from the pharmacokinetic viewpoint, quantification of TA-0201 and its major metabolites in plasma and target tissues is essential. Recently, great progress in the field of mass spectrometry has resulted in development of new techniques such as tandem mass spectrometry (MS/MS) and electrospray ionization (ESI) which markedly improved detection sensitivity for drugs, and quantification in the order of picograms or femtograms is becoming possible. Therefore, LC–MS/MS has attracted attention as a highly sensitive and specific method, and recently it has been used as the first-choice method for assaying clinical samples [6–8]. In the present study, an LC–ESI/MS/MS method for quantitative determination of TA-0201 and its metabolites was developed and applied to determine their concentrations in plasma and tissues of the rat after oral administration of a low therapeutic dose of TA-0201.

2. Experimental

2.1. Materials and reagents

HPLC-grade acetonitrile and acetic acid were obtained from Nacalai Tesque, Ltd. (Kyoto, Japan). Reference compounds of TA-0201, its carboxylic acid- and diol-form metabolites (designated as CA and Diol, respectively) and an internal standard (I.S.; [$^2H_6$]TA-0201) were synthesized at the Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd., Japan. Other reagents and solvents were of the commercial analytical grade.

2.2. Instrumentation and conditions

An HPLC system consisting of a pump (Model 600MS, Waters, MA, USA), an auto-injector (Model 717, Waters) and a TSK-gel ODS-80Ts column (4.6 × 150 mm, 5 μm; TOSOH Co., Ltd., Tokyo, Japan) was used. As the mobile phases, 1% CH_3COOH (A) and acetonitrile (B) were used and stepwise gradient elution was run with: A/B ratios of 70/30, 30/70 and 28/72 at 0, 3 and 8 min, respectively, at the flow rate of 1.0 ml min^{-1}. The column temperature was maintained at 40°C. A TSQ700 triple-stage quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an ESI source was used in the positive-ion selected reaction monitoring (SRM) mode. In ESI–MS, a potential of 4.5 kV was applied to the ESI needle electrode. The temperature of the heated capillary was maintained at 250°C. ESI–MS was carried out using nitrogen as the sheath and auxiliary gases under conditions of the 80 psi and 20 l min^{-1}, respectively, to assist

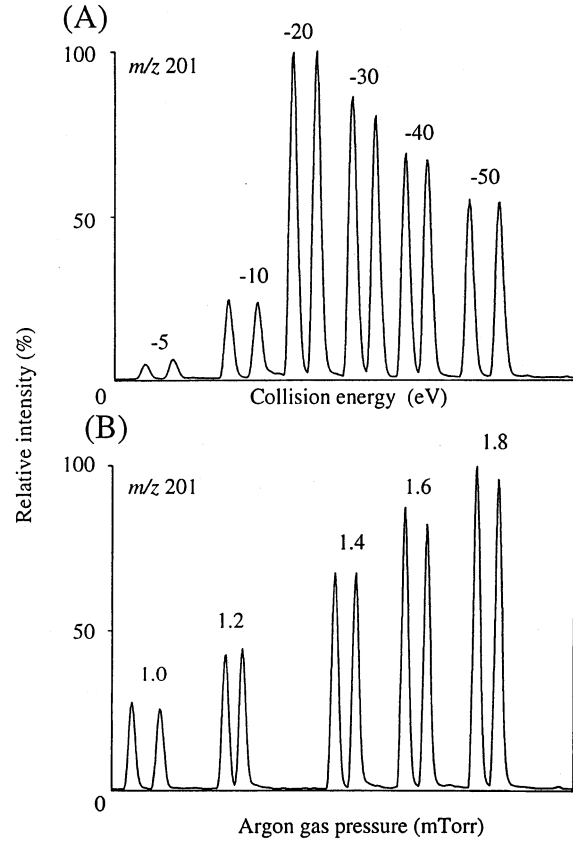


Fig. 3. The effect of collision energy and collision gas pressure on the sensitivity of TA-0201 SRM analysis. (A) Collision energy (eV); and (B) collision gas pressure (m Torr).

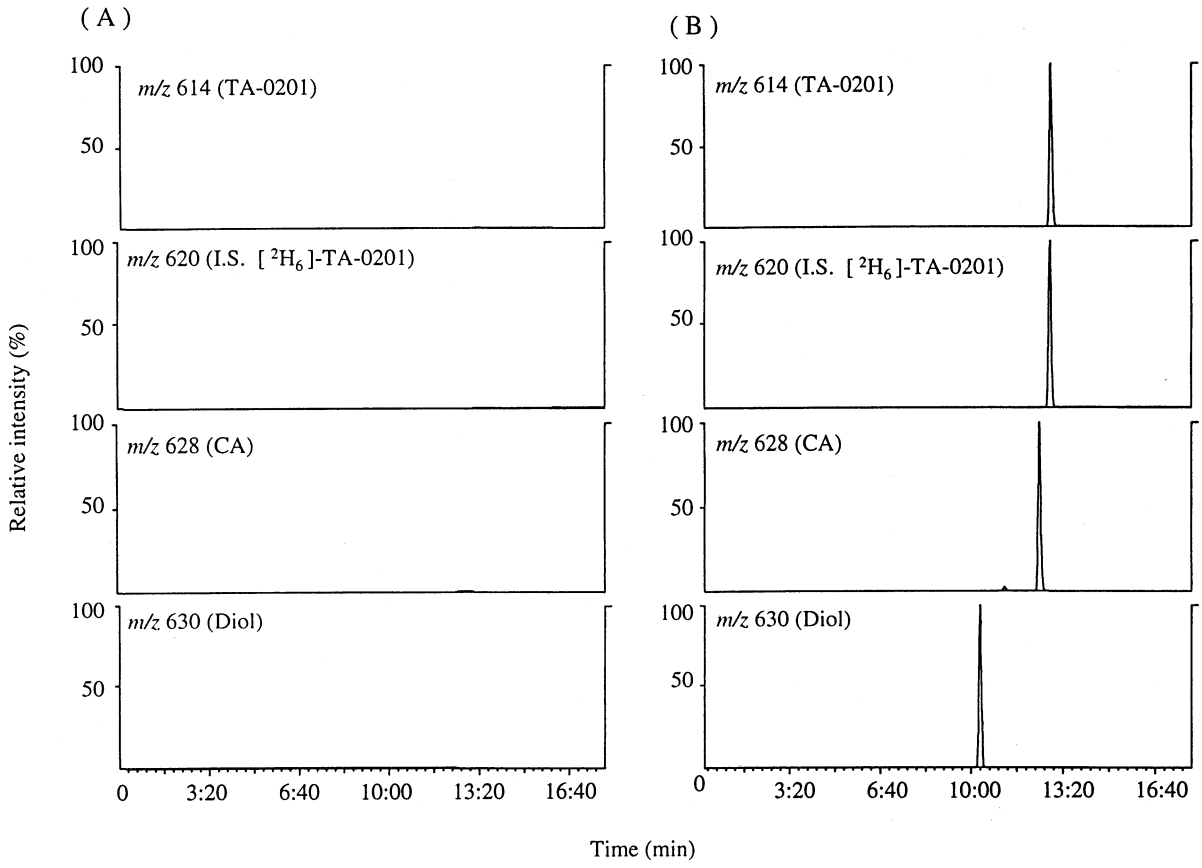


Fig. 4. SRM chromatograms of TA-0201, CA, Diol and I.S. extracted from rat plasma. (A) Blank plasma; and (B) plasma sample spiked with TA-0201, CA, Diol and I.S.(1 ng/0.5 ml each).

nebulization. Precursor ions, i.e. m/z 614, 628, 630 and 620 for TA-0201, CA, Diol and I.S., respectively, were selected by the first analyzer (Q1), then the precursor ions were dissociated by interaction with argon in the collision cell (Q2) and the product ion, m/z 201 in common with all analytes was monitored in the SRM mode in the second analyzer (Q3). Collision-induced dissociation (CID) was carried out using argon at a pressure of 1.8×10^{-3} Torr and with a collision energy of -20 eV.

2.3. Sample preparation

To each biological sample (plasma, 0.5 ml; tissue homogenate, 2 ml) were added 20 μl (10 ng) of I.S. solution (500 ng ml^{-1} in acetonitrile) and 0.5 ml of 1 N HCl and the mixture was extracted with *tert*-butyl methyl ether. After shaking for 10 min and centrifugation at 3500 rpm for 10 min at 4°C, the organic layer was evaporated to dryness at 60°C under a stream of nitrogen, and the residue was dissolved in 0.2 ml of the mobile phase for HPLC, filtered with a membrane filter (0.45 μm), and 0.1 ml of the filtrate was injected into the LC–MS/MS system.

2.4. Preparation of calibration curves

Plasma (0.5 ml) and tissue homogenates (2 ml) from control rats spiked with TA-0201, CA, Diol (0.1, 0.5, 1, 5, 10 or 50 ng each) and I.S. (10 ng) were treated and analyzed by the procedure described above to obtain the calibration curves.

Each calibration curve was drawn by plotting the peak area ratios (TA-0201, CA or Diol to I.S.) versus the concentration of each compound.

2.5. Determination of TA-0201 and its metabolites in plasma and the tissues

Male Sprague–Dawley rats (Charles River Japan, Tokyo), 8 weeks of age and weighing 280–300 g, were used. Animals were fasted overnight before drug administration. Food was withheld until 8 h after administration, but water was available throughout the course of study. TA-0201 (0.1 mg kg^{-1}/5 ml in 0.25% CMC) was administered by gavage to three rats per group. Blood samples were withdrawn with a heparinized syringe from the jugular vein 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration. Plasma was separated by centrifugation at 3500 rpm for 10 min at 4°C. To collect tissues (heart, lung and kidney), rats were anesthetized with diethyl ether 0.5, 4, 24 and 72 h after administration, and sacrificed by withdrawing blood from the abdominal aorta. Tissues were excised and weighed prior to homogenization.

Tissue homogenate samples were prepared with five volumes of 1/15 M phosphate buffer (pH 7.4) by using a Potter–Elvehjem type homogenizer (Digital Homogenizer, IUC, HI).

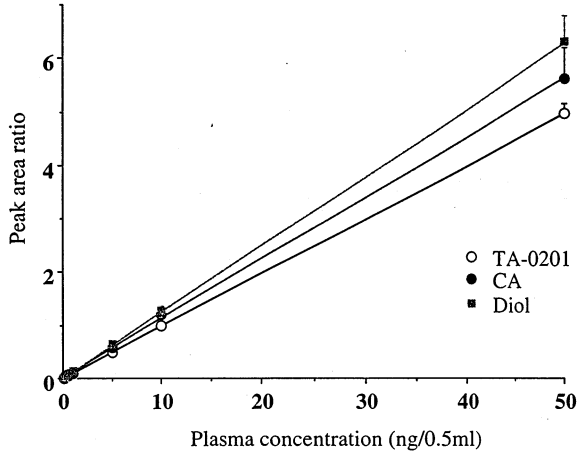


Fig. 5. Typical calibration curves of TA-0201, CA and Diol extracted from rat plasma (0.1–50 ng/0.5 ml). Each point represents the mean ± S.D. of three experiments.

3. Results and discussion

3.1. Conditions for MS/MS

In the product-ion mass spectra of TA-0201, CA, Diol and I.S. (Fig. 2), their protonated molecular ions $[M + H]^+$ were observed at m/z 614, 628, 630 and 620, respectively, and one prominent peak of the characteristic product ion was seen in common at m/z 201 in these spectra. Therefore, these protonated molecular ions were selected by the first analyzer (Q1) as the precursor ions, and the common product ion, m/z 201, was monitored in the second analyzer (Q3) in the SRM mode. Effects of collision energy and collision gas pressure on the sensitivity of the analysis in the SRM mode were investigated by flow-injection analysis using 20 ng of TA-0201 injected twice each time. The intensity of the common product ion, m/z 201, was greatest at the collision energy of -20 eV and the collision gas pressure of 1.8×10^{-3} Torr as shown in Fig. 3. Therefore, these conditions were used to carry out CID.

3.2. Calibration curves

Because our LC–MS/MS method had a high specificity, preparation of the plasma and tissues samples was very simple, requiring only one *tert*-butyl methyl ether extraction under acidic conditions and yielding an almost quantitative recovery (95%).

The mass chromatograms by the SRM method of the samples prepared from control rat plasma (0.5 ml) and the same plasma spiked with 1 ng each of TA-0201, CA, Diol and I.S. are shown in Fig. 4. No peak was observed at the retention times of the respective compounds in the mass chromatograms of control rat plasma (Fig. 4(A)). Since TA-0201, CA, Diol and I.S. were eluted within 13 min as shown in Fig. 4(B), the analysis time for one assay was set at 20 min.

Typical calibration curves for TA-0201, CA and Diol (0.1–50 ng) in rat plasma (0.5 ml) are shown in Fig. 5. Each calibration curve shows good linearity throughout the range of 0.1–50 ng/0.5 ml with a regression coefficient of 1.000, and the lower limits of determination of TA-0201, CA and Diol

Table 1
Precision and accuracy of SRM analysis of TA-0201, CA and Diol in rat plasma[a]

Compound	Concentration (ng/0.5 ml)	Concentration found (mean ± S.D.)	Precision (RSD) (%)	Accuracy (RE) (%)
TA-0201	Intra-day assay			
	0.1	0.098 ± 0.001	1.03	−1.55
	0.5	0.484 ± 0.003	0.67	−3.25
	1	0.985 ± 0.016	1.59	−1.53
	5	4.920 ± 0.249	5.06	−1.59
	10	9.975 ± 0.270	2.71	−0.25
	50	50.047 ± 0.786	1.57	+0.09
	Inter-day assay			
	0.1	0.105 ± 0.001	0.99	+4.67
	0.5	0.493 ± 0.007	1.38	−1.41
	1	1.001 ± 0.007	0.72	+0.13
	5	4.989 ± 0.091	1.83	−0.22
	10	9.962 ± 0.018	0.18	−0.38
	50	50.010 ± 2.183	4.36	+0.02
CA	Intra-day assay			
	0.5	0.521 ± 0.019	3.61	+4.14
	5	4.907 ± 0.388	7.91	−1.86
	50	50.036 ± 2.038	4.07	+0.07
	Inter-day assay			
	0.5	0.516 ± 0.021	4.04	+3.29
	5	5.027 ± 0.172	3.42	+0.54
	50	49.965 ± 3.385	6.77	−0.07
Diol	Intra-day assay			
	0.5	0.499 ± 0.021	4.15	−0.10
	5	4.997 ± 0.093	1.87	−0.06
	50	50.025 ± 3.957	7.91	+0.05
	Inter-day assay			
	0.5	0.473 ± 0.022	4.57	−5.40
	5	5.066 ± 0.272	5.37	+1.32
	50	49.959 ± 3.343	6.69	−0.08

[a] Each point represents the mean ± S.D. of three experiments.

were 0.1, 0.5 and 0.5 ng/0.5 ml, with a regression respectively. Data of the accuracy and precision of inter- and intra-day assays (Table 1) show that this assay method is very consistent and reliable with low relative error (RE) and relative standard deviation (RSD) for TA-0201 and its major metabolites, CA and Diol.

The calibration curves for these compounds in tissue preparations also showed good precision and accuracy as in plasma preparations (data not shown).

3.3. Concentrations in rat plasma and tissues

Time courses of plasma concentrations of the unchanged drug and the two major metabolites (CA and Diol) after oral administration of TA-0201 (0.1 mg kg^{-1}) to rats are shown in Fig. 6. TA-0201 was rapidly absorbed from the digestive tract to reach a C_{max} of 52 ng ml^{-1} 0.25 h after administration and eliminated from the blood stream with a half-life (0.25–4 h) of 1.2 h, its AUC_{inf} being 86 ng h ml^{-1}. The plasma metabolites, CA and Diol, reached their C_{max} of 61 and 2 ng ml^{-1}, respectively, both 0.25 h after administration and declined with half-lives (0.25–4 h) of 1.8 and 3.4 h, respectively, their AUC_{inf} values being 144 and 23 ng h ml^{-1}. The plasma AUC_{inf} and the concentration of CA 8 h after administration were about 1.7 and 5 times greater than those of TA-0201, respectively.

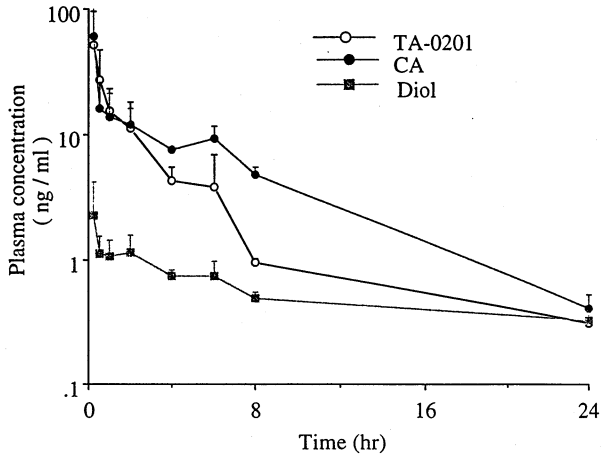


Fig. 6. Plasma concentrations of TA-0201, CA and Diol after oral administration of TA-0201 (0.1 mg kg^{-1}) to male rats. Each point represents the mean ± S.D. of three animals.

Time courses of the concentrations of TA-0201 and its two metabolites in the target tissues of rats after oral administrations at the dose of 0.1 mg kg^{-1} are shown in Fig. 7. In the kidney, lung and heart, TA-0201 reached C_{max} values of 94.5, 80.0 and 33.6 ng g^{-1}, respectively, 0.5 h after administration, and declined more slowly than the plasma concentration of TA-0201 with half-lives of 4–5 h; TA-0201 and CA levels in plasma were below the limits of detection 72 h after administration, while those in the heart (0.4 and 3.3 ng g^{-1}), lung (0.6 and 54.4 ng g^{-1}) and kidney (0.5 and 0.6 ng g^{-1}) were enough to be quantifiable, but Diol levels in these tissues were all less than the limit of detection. CA in these tissues, after reaching its C_{max}, decreased more slowly and its concentrations always remained higher than those of TA-

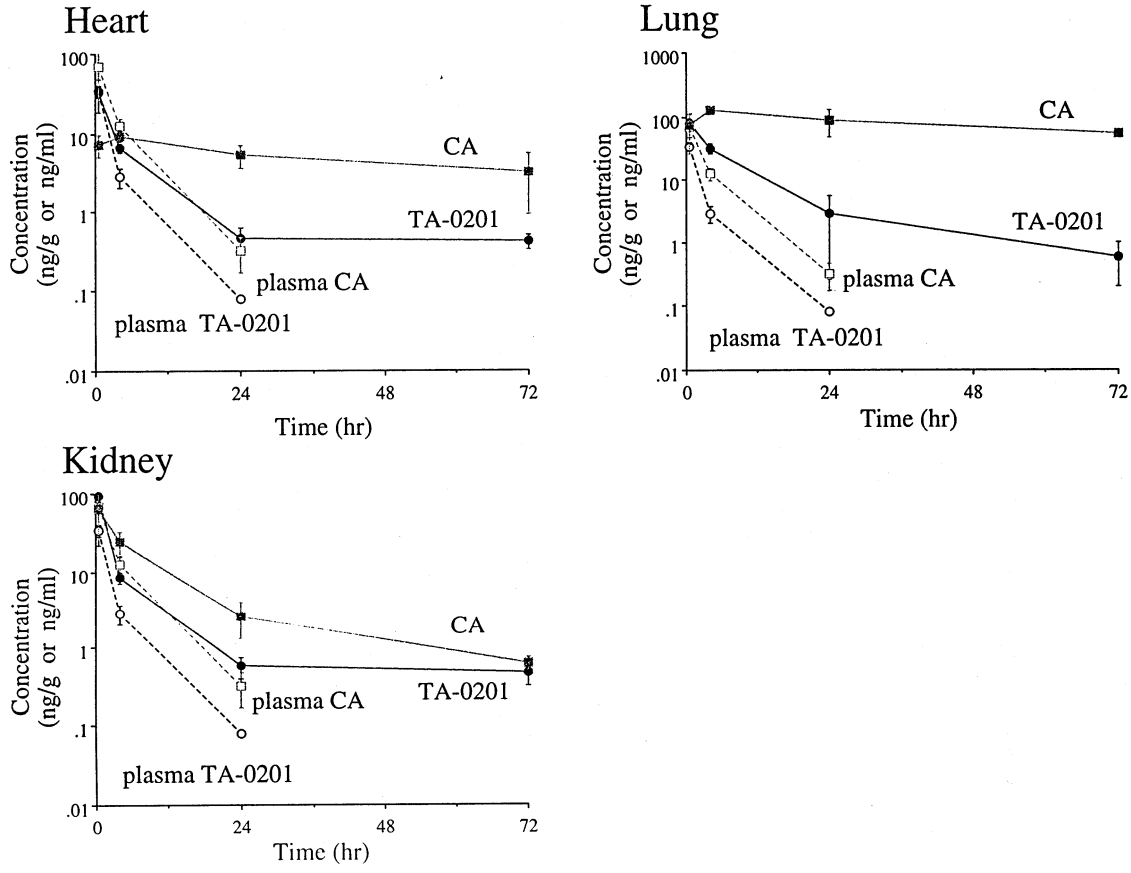


Fig. 7. Tissues concentrations of TA-0201 and CA after oral administration of TA-0201 (0.1 mg kg^{-1}) to male rats. Each point represents the mean ± S.D. of three animals.

0201 for a few days. This tendency was marked in the lung and heart; high CA concentrations were observed 72 h after administration and its AUC_{inf} was 11 272.9 ng h g^{-1}, about 17 times greater than that of TA-0201 (666.3 ng h g^{-1}), in the lung suggesting an extremely high affinity of CA for this tissue.

4. Conclusion

We developed a sensitive, precise and accurate method of simultaneous determination of TA-0201 and its two major metabolites of CA- and Diol-forms using ESI–LC–MS/MS. This method was applied to the determination of these compounds in a pharmacokinetic study with rats. This determination method is considered to be useful enough to measure the plasma concentrations of TA-0201 and its metabolites in clinical pharmacokinetic studies.

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