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Highly sensitive determination of TS-962 (HL-004), a novel acyl-CoA:cholesterol acyltransferase inhibitor, in rat and rabbit plasma by liquid chromatography and atmospheric pressure chemical ionization-tandem mass spectrometry combined with a column-switching technique

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Abstract

A quantitative bioanalytical method with excellent specificity using liquid chromatography (LC) atmospheric pressure chemical ionization-tandem mass spectrometry (APCI-MS–MS) combined with a column-switching technique has been developed for the highly sensitive and reliable determination of TS-962 (HL-004), a novel acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor, in rat and rabbit plasma. The method involves protein precipitation of a 25-μl aliquot of plasma sample with eight volumes of methanol containing a deuterium-labeled internal standard, the direct injection of a methanolic supernatant into the analytical instrumentation with no sample evaporation and reconstitution steps, automated on-line clean-up on a C_{18} short trapping column (10 mm×4.0 mm I.D.) followed by separation on a C_{18} analytical column (50 mm×4.6 mm I.D.), and detection with APCI-MS–MS using m/z 448 ($[M+H]^+$) as a precursor ion and m/z 178 as a product ion in a selected reaction monitoring mode. The lower limit of quantification was 1 ng/ml, and good linearity of the calibration graph was obtained in the range of 1~490 ng/ml with excellent reliability. The developed method enabled pharmacokinetic profiles to be determined for rats and rabbits with sequential plasma collection from an individual animal.

Keywords: Acyl coenzyme A:cholesterol acyltransferase; TS-962

1. Introduction

Accumulation of cholesterol ester on arterial walls is an integral process in atherosclerosis, in which acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes the intracellular esterification of cholesterol [1,2]. TS-962 (HL-004, Fig. 1A), *N*-(2,6-diisopropylphenyl)tetradecylthioacetamide, has been shown to potently inhibit ACAT and to depress the progression of atherosclerotic lesions in laboratory

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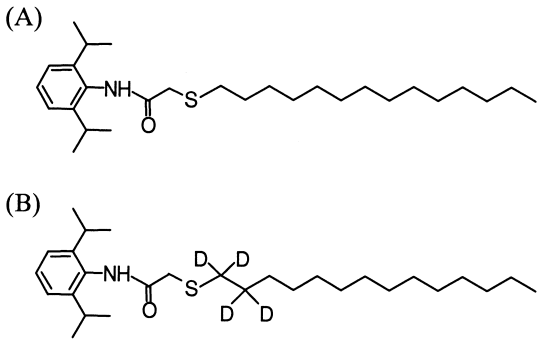


Fig. 1. Chemical structures of (A) TS-962 and (B) TS-962-D_4 (I.S.).

animals [3–5]. It is thus expected to be useful as a new therapeutic drug for atherosclerosis.

A sensitive and reliable bioanalytical method, which is able to determine low nanogram amounts of TS-962 per milliliter of plasma, is required to determine the pharmacokinetic profiles of TS-962 in laboratory animals. An initial attempt was made to develop a method for bioanalytical assay by gas chromatography with electron ionization mass spectrometry [6]. However, this approach required a tedious two-step solid-phase extraction procedure, which was time-consuming and labor-intensive. This method also has the drawback that small rodents such as rats must be sacrificed to obtain large quantities of plasma sample due to its low sensitivity. Accordingly, increased sensitivity must be achieved to decrease the sample volume for quantitative bioanalysis.

Liquid chromatography (LC) coupled with atmospheric pressure chemical ionization tandem mass spectrometry (APCI-MS–MS) has been extensively used for trace-level quantification of drugs in several biological matrices because of its outstanding sensitivity and selectivity [7,8]. This technique was considered to meet our analytical requirements.

The present study was undertaken to develop a sensitive and reliable bioanalytical method for the determination of TS-962 in rat and rabbit plasma by LC–APCI-MS–MS combined with a column-switching technique, which would permit elucidation of plasma concentration–time profiles of this compound with sequential plasma collection from an individual animal.

2. Experimental

2.1. Chemicals

TS-962 and TS-962-D_4 (Fig. 1B), as an internal standard (I.S.), were synthesized at the Pharmaceutical Research Laboratories of Taisho Pharmaceutical Co., Ltd. (Saitama, Japan). Acetonitrile and methanol were of HPLC grade and from Wako Pure Chemical Industries (Osaka, Japan). Distillated water (H_2O) of HPLC grade was purchased from Kanto Chemicals (Tokyo, Japan).

2.2. Blank plasma

Blank plasma samples with heparin as an anticoagulant from male Wistar rats and male New Zealand white rabbits were purchased from Nihon SLC (Shizuoka, Japan). The blank plasma was stored at approximately –20°C until use.

2.3. LC–APCI-MS–MS

APCI-MS–MS with positive-ion detection mode was performed with a VG Quattro-II triple-stage quadrupole tandem mass spectrometer equipped with an APCI source (Micromass, Altrincham, UK). The ion source temperature was set at 150°C, and APCI was performed at a corona discharge voltage of 3.2 kV with a vaporizer temperature of 400°C, a sheath gas (N_2), flow-rate of 150 l/h, and a drying gas (N_2), flow-rate of 275 l/h. Selected reaction monitoring (SRM) was performed with a dwell time of 0.5 s for each target compound with a collision gas (Ar) pressure of 1.8×10^{-3} mbar and a collision energy of 20 eV. The precursor ion was m/z 448 for TS-962 and m/z 452 for the I.S., and the product ion was m/z 178 for both target compounds. Instrument control, data acquisition, and data processing were performed with a Micromass Mass Lynx 2.22 computer software (Micromass). The APCI-MS–MS system was combined with the following column-switching LC system.

2.4. Column-switching LC

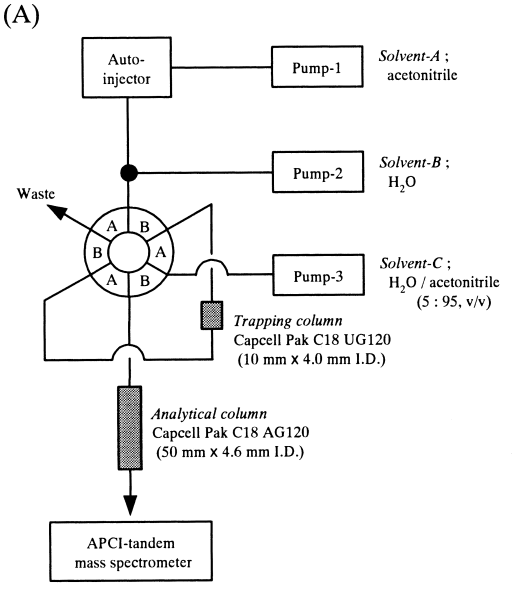
The column-switching LC system was constructed with three pumps [pumps 1 and 3: Hitachi L-6200 (Tokyo, Japan), pump 2: Shimadzu LC-9A (Kyoto, Japan)], a JASCO HV-992-01 six-port switching valve (Tokyo, Japan), and a JASCO 851-AS auto-injector arranged as shown in Fig. 2A. The columns used in the system were a Shiseido Capcell Pak C_{18} UG120 short-trapping column (5 μm, 10 mm×4.0 mm I.D., Tokyo, Japan) and a Shiseido Capcell Pak C_{18} AG120 analytical column (3 μm, 50 mm×4.6 mm I.D.). These columns were maintained at 50°C with a TOSOH CO-8010 column oven (Tokyo, Japan).

As the mobile phase for directing the sample solution to the trapping column and column refreshing, solvent A (acetonitrile delivered from pump 1) and/or solvent B (H_2O delivered from pump 2) were used. With use of pump 2, a post-injector dilution of solvent A with solvent B was performed. Solvent C [H_2O–acetonitrile (5:95, v/v)] was used as the mobile phase for the analytical column and was delivered from pump 3 at a flow-rate of 1.0 ml/min.

The time program for the column-switching system is summarized in Fig. 2B. After injection of a 100-μl aliquot of the sample solution using the autoinjector, the sample was directed to the trapping column with a trapping solution [solvent A–B (40:60, v/v)] at a flow-rate of 1.0 ml/min, followed by flushing by further elution with the same mobile phase for 1 min. The valve was switched following cessation of delivery of the trapping solution to deliver the analyte from the trapping column into the analytical column by eluting solvent C for 1.5 min. The switching valve was then turned back to the initial position and the trapping column was subjected to flushing by eluting solvent A at a flow-rate of 1.5 ml/min for 1.5 min to wash out any retained endogenous compounds and re-equilibrated with the trapping solution for 2 min.

2.5. Standard solutions

TS-962 was dissolved in methanol to prepare a primary stock solution (2.000 mg/20 ml), and a series of working standard solutions containing TS-962 (0.5~250 ng/10 μl in methanol) was prepared



(B)

Position of the switching valve	B	A	B	
Mobile phase for the trapping column	Trapping solution[a]	Solvent-C	Solvent-A	Trapping solution[a]
Flow-rate(ml/min) at the trapping column	1.0	1.0	1.5	1.0
State of the trapping column	Enrichment	Transfer	Refreshing	Equilibration
State of the analytical column	Idling	Separation		
Flow-rate(ml/min) at the analytical column	1.0			
Mobile phase for the analytical column	Solvent-C			

0.0 1.0 2.5 4.0 6.0 (min)

[a] Trapping solution: Solvent-A/B (40 : 60, v/v)

Fig. 2. (A) Schematic diagram and (B) time schedule of the column-switching system.

from the primary stock solution. A working I.S. solution (0.2 ng/200 μl) was also prepared by diluting the primary stock solution for the I.S. (2.000 mg/20 ml) with methanol. All standard solutions were stored at 4°C.

2.6. Preparation of calibration and QC samples

For preparation of calibration and validation of QC samples, a 10-μl aliquot of the respective working standard solution was spiked into 500-μl aliquots of rat and rabbit blank plasma. The resulting plasma concentrations were in the range of 1~490 ng/ml. The stability QC samples (1, 25, and 490 ng/ml) were prepared in the same manner except for an increase in sample volume to be prepared.

2.7. Sample work-up

A 200-μl aliquot of the working I.S. solution was mixed with a 25-μl aliquot of plasma, and the entire mixture was shaken for 10 min. After centrifugation at 2056 *g* for 5 min, the supernatant was filtered with a Millipore Ultrafree MC tube (0.2 μm, Tokyo, Japan). The resulting filtrate was transferred to an autoinjector vial, and a 100-μl aliquot of the methanolic sample was directly injected into the analytical instrumentation.

2.8. Calibration graph

The calibration graph was constructed by plotting the ratio of the peak area of TS-962 to that of the I.S. (8 ng/ml) versus the concentration of TS-962 (1, 5, 25, 98, and 490 ng/ml) using weighted ($1/y^2$) linear regression.

2.9. Precision and accuracy

The precision and accuracy of the developed method were evaluated by analyzing three validation QC samples (1, 25, and 490 ng/ml) in replicates of five on three separate days. All the concentrations of TS-962 were determined using the calibration equation.

2.10. Recovery

The extraction efficiency of TS-962 from rat plasma was determined by comparing the TS-962–I.S. peak area ratios obtained from two kinds of recovery samples. One was the validation QC sample spiked with the I.S. after the extraction. The other was the blank plasma sample spiked with both TS-962 and the I.S. after the extraction. Evaluation of the recovery was performed in replicates of five at each of three concentrations (1, 25, and 490 ng/ml). The recovery for the I.S. was assessed at 8 ng/ml in the same manner.

2.11. Stability

The long-term storage stability of TS-962 in both rat and rabbit plasma was assessed by examining the stability of the QC samples (1, 25, and 490 ng/ml) immediately after their preparation and after being stored at approximately –20°C for 3 or 4 months. To evaluate stability during sample freezing and thawing, the stability of the QC samples were analyzed after three freeze–thaw cycles. The assay values were compared with those obtained from the spiked sample without freezing.

3. Results and discussion

3.1. Method development

A highly sensitive and reliable quantitative bioanalytical method, making possible sequential plasma collection from an individual animal is required to conduct pharmacokinetic studies on TS-962 in rats and rabbits. LC–APCI-MS–MS is now widely used for pharmaceutical bioanalysis, owing to its outstanding sensitivity and selectivity. Since endogenous compounds in biological matrices can severely affect the assay sensitivity and raggedness, suitable sample pretreatment prior to LC–APCI-MS–MS analysis is needed to fully benefit from the use of this technique. Plasma pretreatment has typically been performed using liquid–liquid extraction, solid-phase extraction, and protein precipitation. Of these techniques, protein precipitation with a water-miscible organic solvent, such as methanol and acetoni-

trile, is very useful because of its simplicity and wide applicability. Since a plasma sample undergoes dilution in the protein precipitation, sample preconcentration steps involving evaporation and reconstitution are often required prior to analysis, but are tedious and time-consuming. In addition, this results in loss of analytes due to adsorption to the surfaces of the devices, especially when highly lipophilic compounds such as TS-962 are analyzed. Therefore, on-line clean-up with direct-injection of a methanolic supernatant in protein precipitation may be strongly preferable for the sensitive high-throughput quantitative analysis of TS-962 in rat and rabbit plasma. We therefore attempted to develop a method employing LC–APCI-MS–MS combined with a useful column-switching technique.

The APCI full scan mass spectrum of TS-962 showed an abundant ion at m/z 448 as a $[M+H]^+$, which underwent extensive fragmentation by collision-induced dissociation (CID) to give an abundant product ion at m/z 178 corresponding to the 2,6-diisopropylphenylamine moiety of the molecule (Fig. 3A). The intensity of the product ion at m/z 178 was maximized at a collision energy of 20 eV and a collision gas pressure of 1.8×10^{-3} mbar. Based on these observations, the precursor/product combination of m/z 448→178 under the described CID conditions was selected.

In the mass spectrometric determination of trace compounds in biological matrices, use of a stable isotope-labeled compound as an I.S. is recommended for performing a reliable assay. In the SRM mode, an I.S. which gives the same product ion with a target analyte is much preferred. Therefore, TS-962 having four deuterium atoms at the $-S-C_{14}H_{29}$ side chain of the molecule was designed and used as the I.S. The APCI product ion mass spectrum is depicted in Fig. 3B.

For on-line clean-up using a column-switching technique, it is very important to retain the analyte on a trapping column, which removes a large excess of coexisting compounds. Our previous study demonstrated a simple column-switching system equipped with a by-pass tube for the direct-injection of a large volume of a methanolic sample, which is constructed with a short trapping column, an analytical column, a six-port switching valve, and an autoinjector [9]. With the use of the by-pass tube,

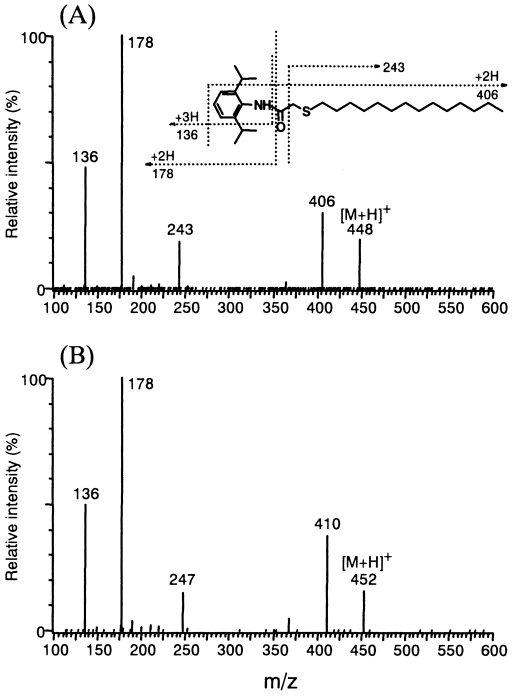


Fig. 3. APCI product ion mass spectra of (A) TS-962 and (B) the I.S.

which splits a mobile phase to direct a sample solution to the trapping column at a ratio of 1:9, 90% of the mobile phase flow passes the autoinjector, and the separated mobile phases are mixed continuously before reaching the switching valve connected to the trapping column. In this system, however, a solution with lower eluting strength must be used as a mobile phase for entry into the autoinjector in order to enrich the analyte onto the trapping column. This may not be probably preferable for analysis of the highly lipophilic compound TS-962 because of the possibility of analyte adsorption within the injection system, such as rotor seals, and inner walls of the sample-loop and tubing [10]. In addition, the by-pass system maintains steady mixing conditions only if the pressure drop in the two flow paths between two T-pieces, which are fitted upstream and downstream of the injector, remains constant. Therefore, in the present study, a column-switching system using acetonitrile as a mobile phase for entry into an autoinjector was constructed, in which post-injector addition of H_2O delivered from an additional pump

(pump 2) into the acetonitrile flow was performed to decrease its eluting strength (Fig. 2A). When TS-962 with 100 μl of methanol was delivered to the trapping column without any dilution, it was hardly retained on the trapping column (Fig. 4A). On the other hand, a suitable 2.5-fold dilution of the mobile phase could be used to complete enrichment (Fig. 4B).

To obtain a satisfactory specificity with a minimal analytical run time, optimal separation conditions such as column length and mobile phase composition were determined. Excellent separation was successfully obtained under the conditions described in Section 2. Figs. 5B and 6B show typical SRM chromatograms of TS-962 and the I.S. spiked in rat and rabbit blank plasma, respectively, at 1 ng/ml, which was set as the lower limit of quantification.

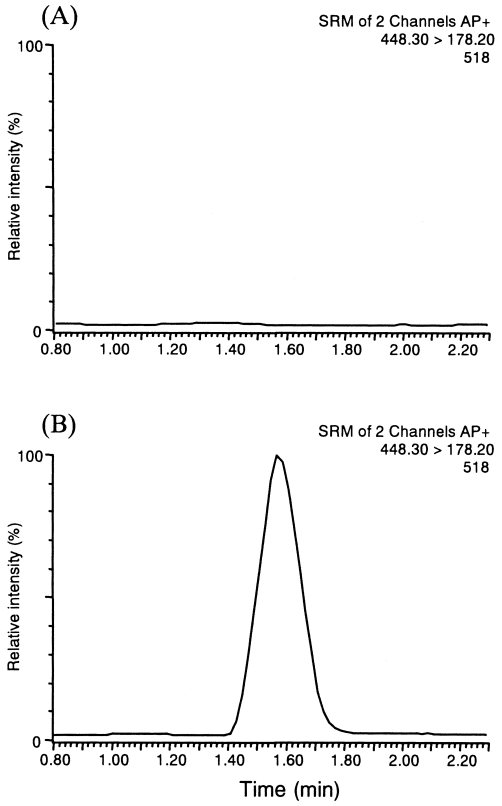


Fig. 4. Enrichment of TS-962 onto the trapping column (A) without and (B) with post-injector dilution of acetonitrile as a mobile phase to decrease its eluting strength. A 100-μl aliquot of TS-962 in methanol (0.05 ng/100 μl) was delivered to the trapping column with acetonitrile at a flow-rate of 0.4 ml/min. After 1 min, the switching-valve was switched to transfer the analyte from the trapping column to the tandem mass spectrometer.

3.2. Validation study

The validation study of the newly developed method was then undertaken. Initially, to elucidate the specificity of the method, independent blank plasma samples from rats and rabbits (six each) were subjected to analysis. Typical SRM chromatograms from blank rat and rabbit plasma are shown in Figs. 5A and 6A, respectively, in which no interference in either matrix was observed. Thus, the method was proven to exhibit excellent specificity.

The linearity of the calibration graph was observed in the range of 1~490 ng/ml. This range permitted the determination of TS-962 in both rat and rabbit plasma obtained in pharmacokinetic studies. Moreover, when the calibration graphs were determined on 3 separate days, the accuracies (percent of nominal concentration) for the mean back-calculated values were within 98.7~102.3% for all the concentrations, indicating excellent linearity (Table 1). Typical parameters for the calibration graph were as follows: slope=0.1104, intercept=0.0100, and correlation coefficient=0.9994. The intercept of the calibration graph was nearly equal to zero, demonstrating excellent specificity of the method as well as no interference by non-labeled TS-962 contained in the I.S.

The recovery of TS-962 from rat plasma was found to be between 74.2 and 76.9% within a C.V. of 7.9% in all the concentration ranges, which was equivalent with that of the I.S. The analyte recovery from rabbit plasma was not determined, but it would be probably similar to that in rat plasma since the assay sensitivity is equivalent for the two matrices.

The precision and accuracy were further examined by analysis of spiked plasma samples with concentrations of 1, 25, and 490 ng/ml (Table 2). The intra-day and inter-day precisions (C.V.%) were within 11.2 and 7.8%, respectively, and intra-day and inter-day accuracies were within the range of 95.5~106.8%, demonstrating very high reliability of the method.

Finally, stability evaluation of TS-962 was carried out. The stability of TS-962 in the standard working

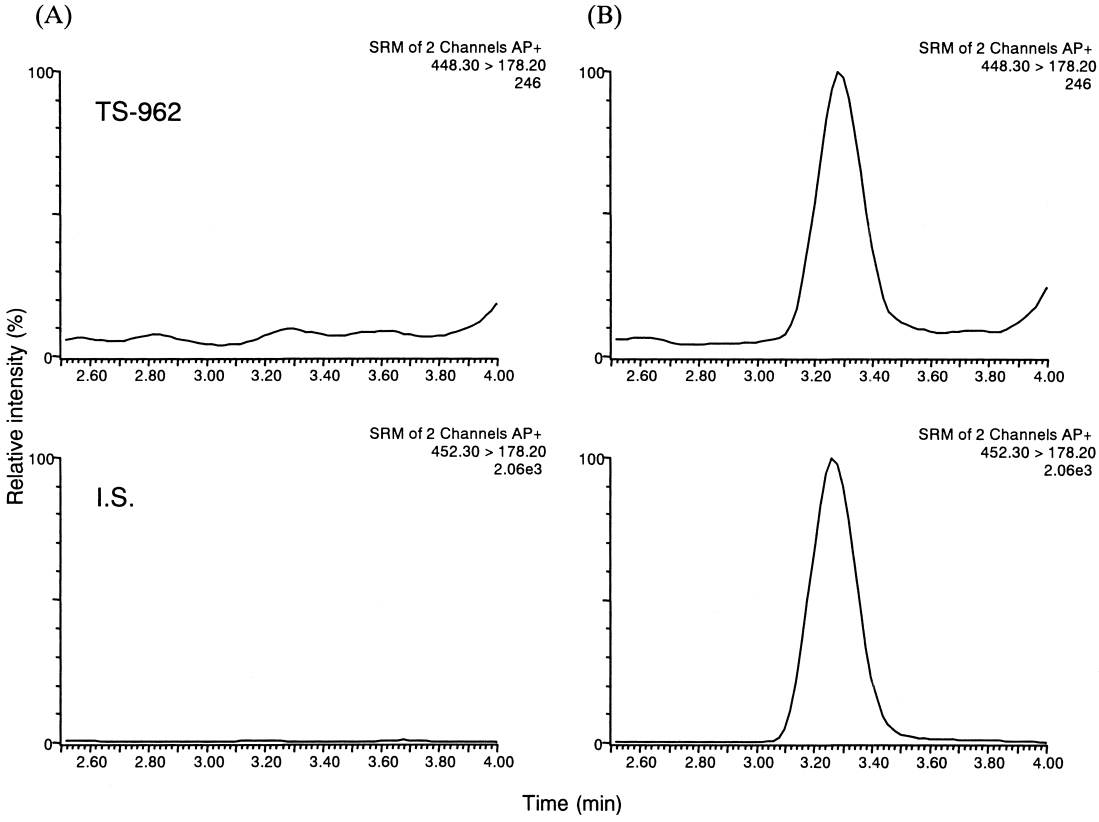


Fig. 5. Typical SRM chromatograms of TS-962 and the I.S. in (A) blank rat plasma and (B) spiked rat plasma with a TS-962 concentration of 1 ng/ml with 8 ng/ml of the I.S.

solution at 4°C was examined using three levels of the methanolic solution with concentrations of 0.5, 12.5, and 250 ng/10 μl. As a result, no changes were found for at least 189 days, and there was no evidence of degradation. A similar result was obtained for the working I.S. solution. Analyte stability in rat and rabbit plasma at concentrations of 1, 25, and 490 ng/ml was further examined. TS-962 in both matrices demonstrated acceptable stability after storage at approximately −20°C for 3 or 4 months (Table 3) and over three freeze–thaw cycles (Table 4).

3.3. Method application

The developed method was applied to the pharmacokinetic studies of TS-962 in rats and rabbits. The results obtained with a rat after oral administration of 1 mg/kg are presented in Fig. 7. As expected, the method enabled us to completely follow the concentration–time course up to 24 h after administration, with sequential plasma collection from an individual rat. The outstanding advantage of this method is the small number of animals and reduced time required for analysis.

4. Conclusion

The utility of LC–APCI-MS–MS combined with a column-switching technique for the highly sensitive determination of TS-962 in rat and rabbit plasma was demonstrated. The developed method involves protein precipitation of plasma samples with methanol containing a stable-isotope-labeled I.S., direct injection of the resulting methanolic supernatant into a

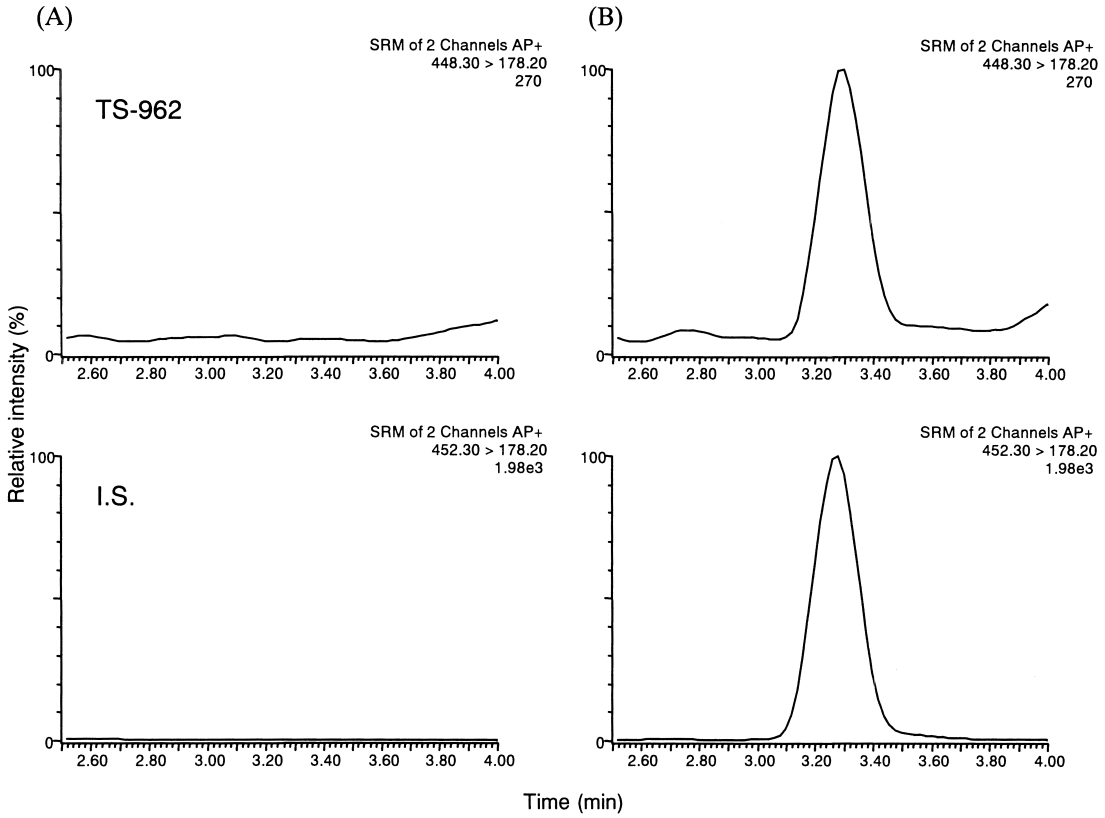


Fig. 6. Typical SRM chromatograms of TS-962 and the I.S. in (A) blank rabbit plasma and (B) spiked rabbit plasma with a TS-962 concentration of 1 ng/ml with 8 ng/ml of the I.S.

C_{18} short trapping column and subsequent automated on-line clean-up, and separation on a C_{18} analytical column followed by detection with APCI-SRM. This novel method was applied to the pharmacokinetic profiling of TS-962 from a preclinical study with sequential plasma collection from an individual animal after oral administration of the compound. It is hoped that availability of a method for the determination of trace amounts of TS-962 in plasma will provide much more precise knowledge con-

Table 1
Back-calculated concentrations from the calibration graphs over 3 days

Nominal concentration (ng/ml)	Rat plasma			Rabbit plasma		
	Back-calculated conc. (ng/ml)[a]	C.V. (%)	Accuracy (%)	Back-calculated conc. (ng/ml)[a]	C.V. (%)	Accuracy (%)
0.980	0.977	0.6	99.7	0.978	0.8	99.8
4.902	5.015	3.0	102.3	4.993	4.9	101.8
24.510	24.606	1.6	100.4	24.285	5.1	99.1
98.039	97.362	0.6	99.3	97.115	2.2	99.1
490.196	483.913	1.8	98.7	495.709	1.4	101.1

[a] Each value represents the mean concentration from three experiments.

Table 2
Intra-day and inter-day precision and accuracy for the determination of TS-962 in rat and rabbit plasma

Day	Nominal	Concentration (ng/ml)					
		Rat plasma			Rabbit plasma		
		QC-1	QC-2	QC-3	QC-1	QC-2	QC-3
		0.980	24.510	490.196	0.980	24.510	490.196
1	Found	0.967	24.911	486.098	0.963	22.899	482.446
		0.976	24.009	489.423	0.909	24.960	481.759
		0.933	24.564	476.461	0.988	24.812	498.910
		1.017	23.640	487.893	0.956	24.813	476.959
		1.027	25.210	469.073	0.862	24.824	475.686
	Mean (ng/ml)	0.984	24.467	481.790	0.936	24.462	483.152
	Intra-day precision (%)	3.900	2.627	1.810	5.354	3.580	1.922
	Intra-day accuracy (%)	100.408	99.824	98.285	95.469	99.803	98.563
2	Found	1.012	23.662	490.646	0.981	25.054	480.933
		0.922	25.106	493.770	0.985	25.304	485.943
		1.087	25.810	486.319	0.925	26.921	434.055
		0.804	24.898	515.541	1.036	26.151	484.173
		1.009	24.777	518.792	0.875	26.688	467.586
	Mean (ng/ml)	0.967	24.851	501.014	0.960	26.024	470.538
	Intra-day precision (%)	11.186	3.121	2.999	6.438	3.169	4.596
	Intra-day accuracy (%)	98.653	101.390	102.207	98.000	106.175	95.990
3	Found	0.907	24.436	483.514	1.032	26.254	490.804
		0.973	26.203	472.291	0.876	26.177	482.528
		0.896	24.994	476.832	1.026	26.654	511.711
		1.095	26.959	481.796	0.886	25.135	479.670
		0.929	24.780	483.896	1.029	26.670	477.770
	Mean (ng/ml)	0.960	25.474	479.666	0.970	26.178	488.497
	Intra-day precision (%)	8.440	4.176	1.041	8.370	2.387	2.845
	Intra-day accuracy (%)	97.959	103.935	97.852	98.959	106.805	99.653
	Overall mean (ng/ml)	0.970	24.931	487.490	0.955	25.554	480.729
	Inter-day precision (%)	7.814	3.583	2.843	6.550	4.231	3.445
	Inter-day accuracy (%)	99.007	101.716	99.448	97.476	104.261	98.069

Table 3
Long-term storage stability of TS-962 in rat and rabbit plasma

Sample	Nominal conc. (ng/ml)	Found[a] (% of initial conc.)	
		Rat plasma[b]	Rabbit plasma[c]
QC-1	0.980	86.9	90.0
QC-2	24.510	94.9	98.8
QC-3	490.196	92.8	98.7

[a] Each value represents the mean from three experiments.
[b] The rat plasma sample was subjected to analysis after a 92-day storage period.
[c] The rabbit plasma sample was subjected to analysis after a 120-day storage period.

Table 4
Stability of TS-962 in rat and rabbit plasma after three freeze–thaw cycles

Sample	Nominal conc. (ng/ml)	Found[a] (% of initial conc.)	
		Rat plasma	Rabbit plasma
QC-1	0.980	94.9	93.7
QC-2	24.510	99.7	98.6
QC-3	490.196	93.5	95.2

[a] Each value represents the mean from three experiments.

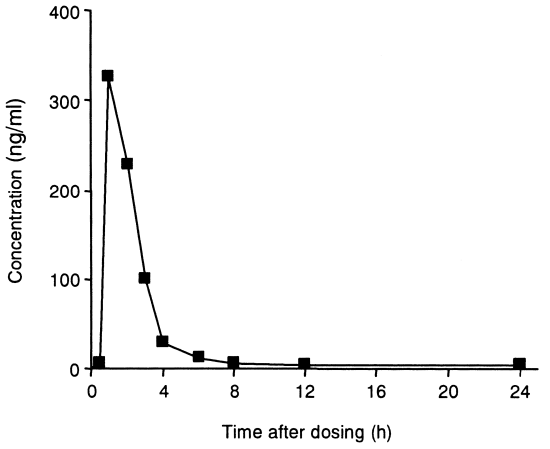


Fig. 7. Typical plasma concentration–time profile of TS-962 in a rat after oral administration of 1 mg/kg.

cerning the pharmacological efficacy of this compound.

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References

[1] K.E. Suckling, E.F. Stange, J. Lipid Res. 26 (1985) 647.
[2] M.S. Brown, Y.K. Ho, J.L. Goldstein, J. Biol. Chem. 255 (1980) 9344.
[3] I. Ishii, N. Yokoyama, M. Yanagimachi, N. Ashikawa, M. Hata, S. Murakami, Y. Asami, N. Morisaki, Y. Saito, S. Ohmori, M. Kitada, J. Pharmacol. Exp. Ther. 287 (1998) 115.
[4] Y. Asami, I. Yamagishi, S. Murakami, H. Araki, K. Tsuchida, S. Higuchi, Life Sci. 62 (1998) 1055.
[5] Y. Asami, I. Yamagishi, K. Akiyoshi, H. Tomoike, K. Tsuchida, S. Higuchi, Atherosclerosis 146 (1999) 237.
[6] J. Kokatsu, Y. Kimura, personal communication.
[7] E.C. Huang, T. Wachs, J.J. Conboy, J.D. Henion, Anal. Chem. 62 (1990) 713A.
[8] E. Brewer, J. Henion, J. Pharm. Sci. 87 (1998) 395.
[9] J. Yamaguchi, Y. Watanabe, M. Ohmichi, S. Jingu, N. Ogawa, J. Kokatsu, K. Fukushima, J. Goto, J. Chromatogr. B 730 (1999) 61.
[10] W.J. Lough, M.J. Mills, J. Maltas, J. Chromatogr. A 726 (1996) 67.