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Novel methylcellulose-immobilized cation-exchange precolumn for on-line enrichment of cationic drugs in plasma

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Abstract

We developed a novel methylcellulose-immobilized strong cation-exchange (MC-SCX) precolumn for direct analysis of drugs in plasma. MC-SCX consists of silica gel with a methylcellulose outer-surface and a 2-(4-sulfophenyl) ethyl phase inner-surface. The MC-SCX precolumn was evaluated by direct analysis using pyridoxine, atenolol and sulpiride spiked in plasma, using a column-switching HPLC system. Each drug was retained and enriched on MC-SCX using an acidic mobile phase, which resulted in good linearity, sufficient reproducibility, intra- and inter day precision, and accuracy in analytical ion-pair LC with trifluoroacetic acid. The analytical methods for model drugs were applied to pharmacokinetics of atenolol and sulpiride in rats.

Keywords: Pharmacokinetics; Pyridoxine; Atenolol; Sulpiride

1. Introduction

High-performance liquid chromatography (HPLC) remains a powerful tool for analysis of small molecules such as drugs and drug metabolites in biological specimens. Direct injection of biological samples such as plasma and urine into HPLC systems coupled with a restricted access media (RAM) column is an attractive alternative to traditional off-line sample preparation techniques. The development of internal surface reversed phase (ISRP) columns by Pinkerton and coworkers [1–3] brought about simplification of the clean-up procedures including direct injection of plasma samples into HPLC systems. Several RAM columns, such as the improved ISRP [4], mixed functional phase silica [5], semipermeable surface silica [6] and diol silica [7,8] have been developed for drug analysis.

A methylcellulose-immobilized reversed-phase (MC-ODS) precolumn [9] is a RAM column with characteristic properties. The external surface of MC-ODS effectively prevents protein adsorption onto the ODS phase while the internal surface of MC-ODS retains small molecules such as drugs and drug metabolites in plasma due to hydrophobic interactions. Moreover, the MC-ODS precolumn possesses good stability in direct injection analysis of plasma using various mobile phases in the pH range of 2–7.

These RAM columns confer some advantages for drug/metabolite analysis in plasma, i.e. simplification of sample preparation and improvement of analytical efficiency. However, column-switching HPLC analysis for hydrophilic drugs using RAM column is still limited by weak retention on the precolumn mentioned above. Therefore, it was important to establish a reproducible method for quantitative determination so a novel RAM material was required.

Recently several reports concerning the use of cation-exchange diol RAM column were reported for analysis of peptides, protein and drugs [10–14]. These reports dealt with the on-line sample clean up of peptides in HPLC-electrospray ionization MS system [10] and the separation of the low-molecular-weight proteinaceous fraction form bio-fluids for protein mapping [11] and size exclu-

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sion and enrichment of low molecular mass proteinaceous samples [12]. Further application for drugs in plasma [13], drugs and their metabolites in blood [14] showed that the cation exchange diol RAM is superior to reversed phase diol-RAM for extraction of hydrophilic basic drugs and peptides.

Herein, we describe development and investigation of direct quantitative analysis of cationic drugs in plasma using a novel methylcellulose-immobilized strong cation-exchange column in column-switching HPLC (LC–LC) system.

2. Experimental

2.1. Chemicals, reagents

Acetic acid, sodium hydroxide and sodium dihydrogenphosphate were purchased from Wako Pure Chemicals (Osaka, Japan). Acetonitrile was purchased from Kanto Chemicals (Tokyo, Japan). TFA was purchased from Pierce (Rockford, IL, USA). All other reagents were analytical grade and used without further purification. Water was deionized and purified by a Milli-Q® purification system from Millipore (Bedford, MA, USA). The model cationic drugs, pyridoxine, atenolol and sulpiride were purchased from Sigma (St. Louis, MO, USA). Structures, dissociation constants (pK_a) [15], and logarithm of octanol–water partition coefficient ($\log P_{ow}$) [16] of drugs are displayed in Fig. 1.

2.2. Animals

Male Sprague–Dawley (SD) rats (7 weeks, 230–287 g) were purchased from Charles River Japan (Yokohama, Japan).

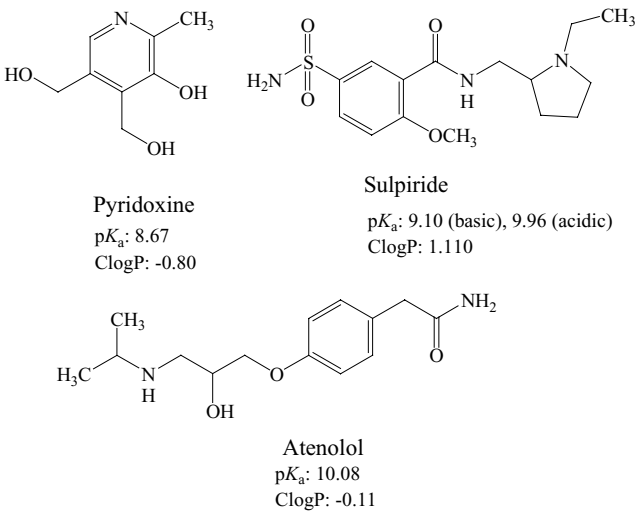

Fig. 1. Structures, dissociation constants (pK_a) and calculated partition coefficients (C log P) of pyridoxine, atenolol and sulpiride.

2.3. Preparation of MC-SCX

Cleansed silica particles (particle size; 50 μm, pore size; 12 nm) were modified with hydrophilic methylcellulose to provide methylcellulose-immobilized silica (MC-silica) by a literature procedure. The internal surface of MC-silica was modified with 2-(4-chlorosulfonylphenyl) ethyltrimethoxysilane (Gelest, PA, USA) using a suitable organic solvent such as cyclohexane. The terminal chlorosulfonyl group of the ligand was hydrolyzed in a solvent, e.g. tetrahydrofuran to the sulfonic acid (sulfonate) group by the addition of water and in this way MC-immobilized strong cation exchange silica (MC-SCX) was obtained.

For the purpose of confirmation of surface modification of silica gel with MC and SCX legand, elemental analysis (C, H, N) for silica gel, MC-immobilized silica and MC-SCX silica were carried out.

2.4. Sample preparation

SD rat plasma was obtained by centrifugation of SD rat blood after anti-coagulate treatment using heparin and used as blank plasma. The drug-containing SD rat plasma samples were prepared by adding pyridoxine, atenolol or sulpiride at the concentrations of 10, 50, 100, 500 and 1000 ng/mL. The calibration standard samples for each drug were dissolved in water at the same concentration as the spiked plasma samples.

2.5. Instrumentation and chromatographic conditions

The HPLC-UV (LC-UV) system consisted of a Shimadzu LC-10ATvp dual pump system, a Shimadzu SPD-10Avp UV detector, Shimadzu SIL-10ADvp auto-injector, a Shimadzu CTO-10A column oven and Shimadzu SCL-10Avp system controller. The analysis was performed using the MC-SCX precolumn (30 mm × 4 mm i.d.) at 30 °C with isocratic elution of a mobile phase of water/acetonitrile (97:3, v/v) containing 0.1% acetic acid and 0, 2, 5, 10, 25 and 50 mmol/L of sodium dihydrogenphosphate at a flow rate of 2.0 mL/ min.

A schematic diagram of the LC–LC–UV system is illustrated in Fig. 2. Samples were injected into the LC–LC–UV system using a Shimadzu SIL-10ADvp auto-injector with on-line one-eighth dilution using a by-pass line. For extraction of cationic drugs, the solutes injected were transferred to the MC-SCX precolumn using an extraction mobile phase at the flow rate of 2 mL/min delivered by a LC-10AT pump.

Following removal of plasma proteins and other matrix components for 2 min, the target drugs, enriched on MC-SCX precolumn, were transferred to the analytical column (L-Column, 150 mm × 4.6 mm i.d., CERI, Tokyo, Japan) by switching a Shimadzu FCV-12AH six-port valve under the gradient elution mode at a flow rate of 1 mL/min at controlled room temperature and detected by a Shimadzu SPD-10Avp UV detector.

The mobile phases were (A) water containing 100 mmol/L of TFA and (B) water/acetonitrile (10:90, v/v) containing

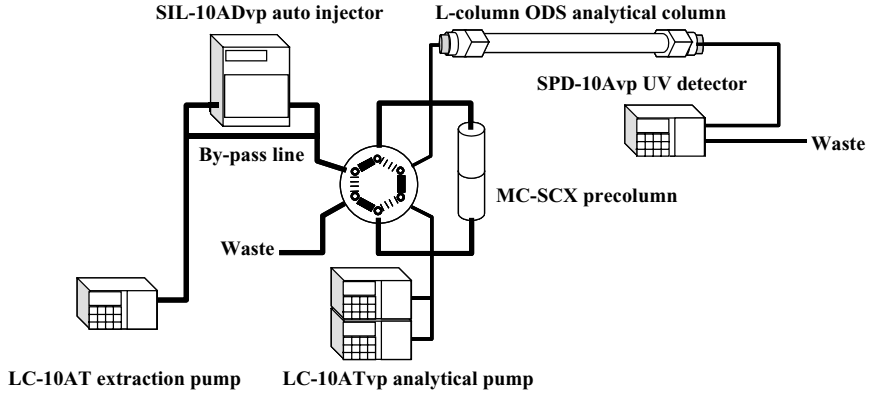

Fig. 2. Schematic diagram of the LC–LC–UV system.

40 mmol/L of TFA, which were delivered by the LC-10ATvp pump (Shimadzu), and the concentration of solvent B was changed gradiently from 0 to 25% over 13 min for pyridoxine and 10 to 25% over 13 min for atenolol analysis, and the isocratic elution using water/acetonitrile (90:10, v/v) containing 40 mmol/L of TFA was employed for sulpiride. The LC chromatogram was monitored on Shimadzu Class VP software ver. 5.1.2 at UV 290 nm for pyridoxine, UV 274 nm for atenolol and UV 242 nm for sulpiride.

2.6. Evaluation of the MC-SCX precolumn

Retention factor of model drugs on the MC-SCX precolumn was determined using LC–UV system for characterization of the MC-SCX precolumn and its batch-to-batch reproducibility using standard drug samples (10 μg/mL).

The effect of co-ion (sodium ion) concentration in the extraction mobile phase on drug recovery was evaluated using LC–LC–UV system. A triplicate injection of the standard drug solution (100 μL, 1000 ng/mL) was made onto the MC-SCX precolumn with an extraction mobile phase of 0.1% acetic acid aqueous solution (pH 3), with or without 10, 20, 50, 75, 100, 300, 500 and 1000 mmol/L of sodium chloride.

The effect of organic solvent concentration in the extraction mobile phase on recovery of drugs in LC–LC–UV system was evaluated. Triplicate injection of a drug-containing plasma sample (100 μL, 1000 ng/mL) was performed onto the MC-SCX precolumn with an extraction mobile phase of aqueous acetic acid solution (0.1%, pH 3), with or without acetonitrile (3, 5 and 10%, v/v).

Ion exchange capability of the MC-SCX precolumn was evaluated using LC–LC–UV system by the relationship between the amount of model drug injected and the peak area in analytical LC with UV 280 nm detection.

2.7. Drug analysis using LC–LC–UV system and validation of the methods

Chromatographic parameters (retention time and tailing factor), and reproducibility of retention time and peak area were determined by the use of standard solutions and plasma samples for each drug in analytical LC in LC–LC–UV system. Reproducibility was assessed by sixtuplicate injection of the calibration standard and plasma samples containing 1000 ng/mL of drug. Linearity of the standard solutions and plasma samples at drug concentrations of 10, 50, 100, 500 and 1000 ng/mL ($n = 3$) was assessed. Intra-day precision and accuracy were determined at every concentration level by a back calculation method and recovery of the drugs from plasma sample ($n = 3$). Inter-day precision and accuracy were assessed for three concentration levels for model drugs.

2.8. Pharmacokinetics of atenolol and sulpiride in rats

Atenolol solution and sulpiride solution were injected intravenously through the femoral vein to three fasted SD rats, respectively (10 mg/kg for atenolol and 0.9 mg/kg for sulpiride). Blood samples (250–300 μL) were drawn from jugular vein using a heparinized syringe at 0.083, 0.25, 0.5, 1, 1.5, 2 and 4 h following the drug administration. The blood was centifugated to make plasma samples, which was stored at −20 °C until analysis. To avoid the influence of anesthetization, rats had been awake during the experiment.

For the quantifications, calibration curve samples for atenolol and sulpiride were prepared at the concentration of 0, 10, 30, 100, 300, 1000, 3000 and 10,000 ng/mL with rat plasma.

Plasma concentration-time data were analyzed by moment analysis. The area under the concentration-time curve (AUC), the volume of distribution at steady state (V_{dss}) and the total body clearance (CL_{tot}) were estimated.

3. Results and discussion

3.1. Evaluation of MC-SCX material and the MC-SCX precolumn

The MC-SCX material was prepared from porous silica particle and the outer surface of silica was modified with MC followed by the internal surface modification

Table 1
Influence of the co-ion concentration (sodium) on retention factor of model drugs on the MC-SCX precolumn

Drug	MC-SCX batch	Co-ion concentration (mmol/L)				
		2	5	10	25	50
Pyridoxine	1	ND	1.79	1.65	1.26	0.85
	2	ND	1.76	1.65	1.24	0.87
Atenolol	1	ND	1.83	1.82	1.43	0.92
	2	ND	1.90	1.80	1.41	0.91
Sulpiride	1	ND	2.00	1.90	1.53	0.97
	2	ND	2.00	1.88	1.53	0.94

ND; not determined.

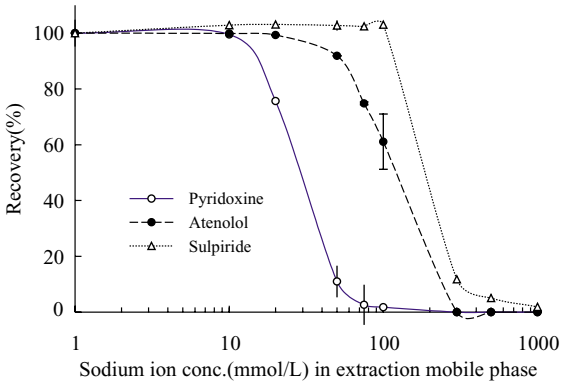

Fig. 3. Effect of co-ion (sodium ion) content in extraction mobile phase on recovery of drugs form the MC-SCX precolumn in the LC–LC–UV system.

with SCX legand. In order to confirm the surface modification, we carried out the elemental analysis for silica gel, MC-immobilized silica and MC-SCX, respectively. As a result, 0.00, 0.14 and 7.44% carbon were found in unmodified silica, MC-silica and MC-SCX, respectively. The increase in carbon content indicated the successful surface modification of silica gel with MC and SCX.

As MC-SCX is cation exchange material, the retention mechanism is based on mainly electostatic interactions. In order to investigate the influence of co-ion concentration on capacity factors of drugs on the MC-SCX precolumn, we determined the retention factor for model drugs with a mobile phase of water/acetonitrile (97:3, v/v) containing 0–100 mmol/L of sodium dihydrogenphosphate (pH 3).

Retention factor of model drugs on the MC-SCX precolumns (two batches) before any injection of plasma sample are shown in Table 1. The MC-SCX precolumn showed that the decrement in retention factor of drugs as the co-ion concentration rises up, which proved the retention mechanism of MC-SCX based on cation-exchange. As for the batch-to-batch reproducibility of MC-SCX, little deference between batches was observed.

The MC-immobilized material can prevent adsorption of plasma protein to the stationary phase under a broad pH range of 2.0–7.0 [9]. We employed acetic acid as a mobile phase additive in extraction LC and TFA in analytical LC in LC–LC–UV analysis. TFA plays an important role firstly, in lowering pH to facilitate desorption of cationic analytes from the ion-exchange site and secondly, the TFA anion forms an ion pair with cationic drugs and thus enables analysis of cationic and hydrophilic drugs using organic solvent rich mobile phases. Retention properties of cationic drugs were evaluated in order to characterize and demonstrate the performance of the MC-SCX precolumn.

Next, we evaluated the influence of the co-ion concentration in the extraction mobile phase on drug recovery from the MC-SCX precolumn in LC–LC–UV system (Fig. 3). An increase in co-ion (sodium ion) concentration in the extraction mobile phase resulted in a reduction in drug recovery from the MC-SCX precolumn. This phenomenon was due to the competition effect of the sodium ion for retention on MC-SCX and the retention strength of drugs on MC-SCX was explained by their basicity (tertiary amine > secondly amine > heterocyclic amine). MC-SCX retains a wide diversity of cationic drugs by using a low ionic strength extraction mobile phase (<10 mmol/L of co-ion). Thus, we employed 0.1% aqueous acetic acid as a mobile phase additive in extraction LC.

The effect of organic solvent in the extraction mobile phase was examined using LC–LC–UV system by assessing the recovery of model drugs from the MC-SCX precolumn using 0.1% aqueous acetic acid solution with acetonitrile (0–10% v/v). In extraction LC using a reversed phase RAM column, a small amount (<10 vol.%) of organic modifier such as methanol and acetonitrile facilitate the release of drugs from the binding site of the plasma proteins [17]. We investigated the effect of acetonitrile concentration on drug recovery from the MC-SCX precolumn in LC–LC–UV system.

It was demonstrated that acetonitrile concentrations (3, 5 and 10%, v/v) of up to 10% had little effect on drug recovery from the MC-SCX precolumn. The actual recovery of model drugs was over 97% and no significant carryover was observed for model drugs even with 0.1% aqueous acetic acid solution as an extraction mobile phase. Hence, we employed 0.1% aqueous acetic acid solution as an extraction mobile phase in further investigation.

The ion exchange capability of the MC-SCX precolumn for model drugs was studied using LC–LC–UV system with 0.1% aqueous acetic acid solution as an extraction mobile phase. Fig. 4 shows the relationship between amounts of samples injected (injection volume; 100 μL) on the MC-SCX precolumn and peak area observed in analytical LC. Though the response was almost linear between 0 and 25 μg of drugs (150 nmol for pyridoxine, 94 nmol for atenolol and 73 nmol for sulpiride), the regression presented a curvature between 0 and 50 μg of drugs. These results indicated that the MC-SCX precolumn has enough ion exchange capability for cationic drugs and its ion exchange efficiency decrease with the increase of drug concentration in the sample solution.

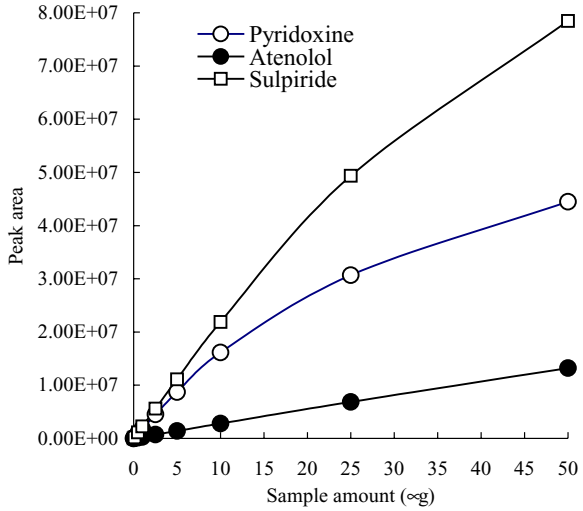

Fig. 4. Relationship between sample amount injected to the MC-SCX precolumn and the peak area observed in analytical LC in the LC–LC–UV system. Detection UV wavelength was 280 nm.

3.2. Drug analysis using LC–LC–UV system and validation of the methods

Direct analysis of pyridoxine, atenolol and sulpiride in plasma was performed with the MC-SCX precolumn in LC–LC–UV system and compared with standard samples. Typical HPLC chromatograms for blank plasma, standard sulpiride and plasma spiked with sulpiride are illustrated in Fig. 5(A–C). Sulpiride was clearly separated from the background peaks, and the chromatographic patterns were reproducible in spite of direct 100 μL injection. The symmetry factor, reproducibility of retention time and target peak area of model drugs were evaluated for both plasma and standard

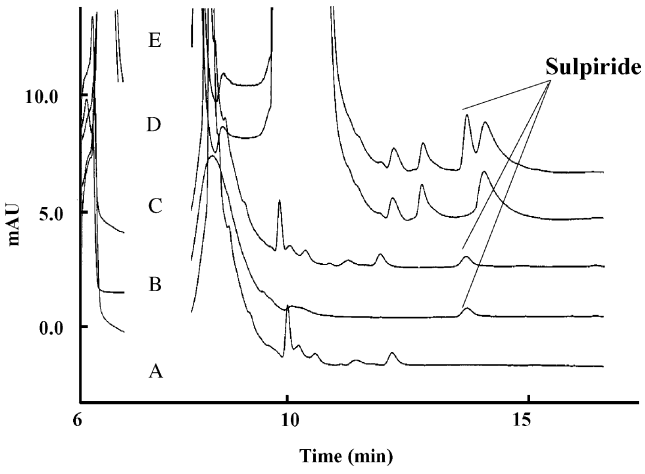

Fig. 5. Typical HPLC-UV chromatograms of sulpiride obtained in the LC–LC–UV system. (A) Plasma blank for spike test. (B) Standard sulpiride (concentration: 50 ng/mL). (C) Plasma spiked with sulpiride: (concentration: 50 ng/mL). (D) Plasma sample at pre- administration. E) Plasma sample at 4 h after 0.9 mg/kg i.v. administration of sulpiride (see Section 2). LC conditions were described in the text.

Table 2
Chromatographic parameters for pyridoxine, atenolol and sulpmde in calibration standard and plasma samples

Drug	Prameter	Calibration standard	Plasma sample
Pyridoxine	Retention time (min)	14.96	15.01
	Symmetry factor	1.28	1.31
	CV (%) for retention time	0.16	0.25
	CV(%) for peak area	0.34	1.01
Atenolol	Retention time (min)	14.95	14.97
	Symmetry factor	1.22	1.12
	CV (%) for retention time	0.10	0.17
	CV(%) for peak area	0.40	1.21
Sulpiride	Retention time (min)	13.34	13.32
	Symmetry factor	1.15	1.11
	CV (%) for retention time	1.20	1.07
	CV(%) for peak area	0.38	0.37

Concentration level; 10 ng/mL for pyridoxine, 100 ng/mL for atenolol and 50 ng/mL for sulpmde. Typical HPLC chromatogram for sulphide is shown in Fig. 5. CV (%) for retention time and peak area; $n = 6$.

samples (Table 2). It was evident that every parameter was almost consistent with those of the standard samples and that the reproducibility of the retention time for sixtuplicate injection was also satisfactory.

The calibration curves ($n = 3$) obtained by peak area versus the drug concentration were linear over the range of 10–1000 ng/mL for every drug. The correlation coefficients (r) were over 0.999 for every drug. The equation of the calibration plot was $y = 2321.8x + 9284.5$ for standard pyridoxine, $y = 2318.9x - 8590.3$ for pyridoxine in plasma, $y = 27.500x - 99.2$ for standard atenolol, $y = 29.200x - 729.1$ for atenolol in plasma, $y = 201.3x - 1755.3$ for standard sulpiride, $y = 206.3x - 1560.7$ for sulpiride in plasma.

Limit of quantitation (LOQ) for the model drugs were assessed with signal to noise ratios and identified as 1, 50 and 10 ng/mL with 100-μL of plasma, for pyridoxine, atenolol and sulpiride, respectively.

The intra- and inter-day precision and accuracy of the methods at different concentrations are shown in Tables 3–5. The intra-day precision ($n = 3$) for plasma sample ranged between 0.03 and 4.49% (CV) for five drug concentrations, while inter-day precision ($n = 6$) ranged between 0.20 and 14.48% at three concentrations. The accuracy of the methods for plasma sample was calculated as percentage of the concentration and the relative error. The relative error calculated as the percent difference of the amount of the drug added to the plasma and the amount found in the experiments ranged form 0.06 to 8.01% for intra-day and 0.46 to 8.42% for inter-day analysis.

These results demonstrate that direct injection analysis of drugs with the MC-SCX precolumn provides a reproducible assay for these cationic drugs in plasma.

On the other hand, column stability is one of the crucial characteristics of RAM columns in routine analysis. We have previously reported the excellent stability of the MC-immobilized reversed phase column. Although rela-

Table 3
Intra-day precisions of the methods for pyridoxine, atenolol and sulpiride in calibration standard and plasma samples

Drug	Concentration (ng/mL)	Calibration standard		Plasma sample	
		Precision	CV (%)	Precision	CV (%)
Pyridoxine	10	7.30 ± 0.2	2.44	13.4 ± 0.1	0.46
	50	48.8 ± 0.2	0.42	50.4 ± 0.5	0.92
	100	102 ± 1.3	1.23	100 ± 0.3	0.30
	500	500 ± 2.1	0.41	489 ± 2.2	0.45
	1000	994 ± 0.7	0.07	999 ± 0.3	0.03
Atenolol	10	3.61 ± 0.0	ND	23.9 ± 0.0	ND
	50	54.6 ± 1.6	2.18	66.4 ± 0.7	1.10
	100	114 ± 2.4	1.83	110 ± 3.2	2.89
	500	540 ± 2.4	0.43	525 ± 2.7	0.51
	1000	1099 ± 7.2	0.65	1106 ± 13.4	1.22
Sulpiride	10	16.1 ± 0.6	1.23	13.7 ± 0.5	3.44
	50	51.9 ± 2.5	2.99	50.2 ± 2.3	4.49
	100	98.0 ± 3.9	2.96	99.4 ± 2.5	2.53
	500	480 ± 25.4	4.95	485 ± 3.8	0.79
	1000	997 ± 13.0	1.26	995 ± 27.4	2.76

CV (%); $n = 3$, ND; Not determined.

Table 4
Intra-day accuracy and the relative error for model drugs in plasma

Concentration (ng/mL)		Drugs		
		Pyndoxine	Atenolol	Sulpinde
10	Accuracy (%)	85.3 ± 0.5	ND	100.0 ± 8.0
	Relative error (%)	0.61	ND	8.01
50	Accuracy (%)	88.4 ± 0.9	88.1 ± 1.5	101.1 ± 5.3
	Relative error (%)	0.99	1.72	5.28
100	Accuracy (%)	90.5 ± 0.3	82.2 ± 3.0	105.3 ± 2.9
	Relative error (%)	0.34	3.66	2.76
500	Accuracy (%)	96.1 ± 0.4	98.7 ± 0.6	103.9 ± 0.8
	Relative error (%)	0.39	0.59	0.81
1000	Accuracy (%)	99.7 ± 0.1	105.0 ± 0.5	102.3 ± 2.9
	Relative error (%)	0.06	0.50	2.80

Relative error (%); $n = 3$, ND; not determined.

Table 5
Inter-day precision and accuracy of the methods in plasma analysis ($n = 6$)

Drug	Theoretical concentration	Precision		Accuracy	
		Observed concentration (ng/mL)	CV (%)	Observed (%)	Relative error (%)
Pyridoxine	10	8.4 ± 1.2	14.48	91.5 ± 6.9	7.51
	100	101.0 ± 2.0	2.00	95.0 ± 5.1	5.39
	1000	994.3 ± 4.0	0.40	99.9 ± 0.5	0.46
Atenolol	55	60.3 ± 3.0	4.9	87.4 ± 1.6	1.83
	100	98.9 ± 9.6	9.71	83.9 ± 2.7	3.26
	1000	1045.6 ± 61.4	5.87	101.8 ± 1.1	1.04
Sulpiride	10	10.9 ± l.l	10.20	93.5 ± 7.9	8.42
	100	99.1 ± 2.0	1.97	100.2 ± 6.0	6.02
	1000	990.0 ± 1.9	0.20	100.0 ± 0.7	0.66

Table 6
Pharmacokinetic parameters for sulpiride and atenolol in SD rats ($n = 3$)

Parameter	Sulpiride	Atenolol
$AUC_{0-\infty}$ (ng/mL)h	357 ± 177	7388 ± 202
V_{dss} (mL/kg)	3536 ± 1180	2795 ± 216
CL_{tot} (mL/min/kg)	48.5 ± 19.4	22.2 ± 0.6

tively high concentrations of TFA were employed in analytical LC, a reduction in chromatographic performance and/or significant pressure build-up in the LC system were not apparent in this study, which involved 300 injections of 100-μL plasma samples over a one-week period.

In addition, we evaluated the durability of the MC-SCX precolumn by retention factor of drugs on the MC-SCX precolumn treated with duplicate injection of 500 μL plasma in LC–UV system. In this study, a mobile phase of water/acetonitrile (97:3, v/v) containing 50 mmol/L of sodium dihydrogenphosphate (pH 3) was employed and on-line dilution for the sample with mobile phase was not made. As a result, a little decrease in the retention factor for drugs was observed for the MC-SCX column compare to that of baseline (see Table 1). Actual retention factor was 0.62, 0.77 and 0.85 for pyridoxine, atenolol and sulpiride, respectively. This phenomenon indicated that a very little plasma protein remained on outer surface of MC-SCX to restrict the drugs from accessing SCX legand on inner surface of MC-SCX and resulted in slight decrease of retention factor for drugs. Nevertheless, in the LC–LC analysis of model drugs, sufficient reproducibility in retention time and peak area, intra- and inter-day precision and accuracy were confirmed as stated above.

These results suggested that the MC-SCX precolumn has enough stability and durability for routine analysis.

3.3. Pharmacokinetics of atenolol and sulpiride in rats

In order to test the applicability of the present method for atenolol and sulpiride, plasma samples obtained from a single intravenously injection to male SD rats ($n = 3$) were analyzed. Typical HPLC chromatograms of plasma samples at pre-administration and at 4 h after 0.9 mg/kg i.v. administration of sulpiride are shown in Fig. 5(D) and (E). In these chromatograms, the background differed from that of spiked sample and its blank sample, however no apparent metabolite peaks was observed in the chromatogram of plasma sample after administration of sulpiride.

Plasma concentration-time profile of sulpiride in rats is shown in Fig. 6 and pharmacokinetic parameters for the both drugs in rats are summarized in Table 6. Pharmacokinetic profile for sulpiride is in general agreement with a report [18] and the pharmacokinetic parameters for atenolol were well coincided literature's value [19].

These results indicated that the MC-SCX precolumn in column switching LC system has been successfully applied to phaemacokinetic studies to quantify atenolol and sulpiride in plasma.

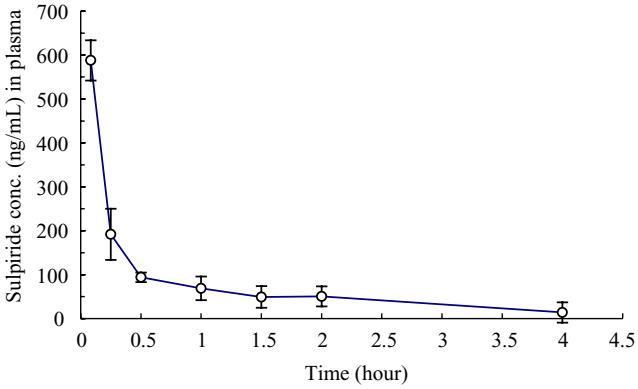

Fig. 6. Plasma concentration–time profile of sulpiride in SD rats.

4. Conclusion

The MC-SCX precolumn was developed and evaluated for direct analysis of cationic drugs in plasma using a column switching LC system. The MC-SCX precolumn proved effective in the on-line enrichment of cationic drugs that have different hydrophobicity and pK_a in plasma sample. These results indicated that the MC-SCX precolumn is applicable to direct analysis of a wide diversity of cationic drugs in plasma. Consequently, the MC-SCX precolumn was found to be a potential tool for pharmacokinetic evaluation of cationic drugs. Further applications of the MC-SCX precolumn and preparation and evaluation of a MC-anion exchange precolumn are currently in progress in our laboratory.

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