Validation of a Column-Switching High-Performance Liquid Chromatographic (HPLC) Method for Determination of ML-1035 and Its Five Metabolites in Plasma

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A fully automated column-switching HPLC procedure has been developed and validated for quantitation of ML-1035 and its five metabolites in plasma employing direct injection. Plasma samples were injected onto a CN extraction column (4 × 4.6 mm, 5 μm) for micellar cleanup with 0.5% sodium dodecyl sulfate (SDS) in 50 m*M* phosphate. The proteinaceous components were solubilized and flushed out. The extracted compounds were then eluted by forward flush onto a C_8 analytical column (150 × 4.6 mm, 5 μm) for further analysis using fluorescence detection (excitation, 308 nm; emission, 350 nm). After the subsequent washing and reequilibration with a sequence of three solvent mixtures, the extraction column was ready for the next injection. The limit of quantitation for all compounds of interest was about 10 to 15 ng/ml using 100 μl of plasma. Excellent precision, accuracy, and linearity were obtained for all compounds over a range of 10 to 1500 ng/ml. The practicality of the HPLC method was also validated with plasma samples from dogs receiving ML-1035. Longevity for both extraction and analytical columns is excellent. Micellar cleanup coupled with the column-switching technique is a promising HPLC procedure when using direct injection of biological fluids.

KEY WORDS: column-switching high-performance liquid chromatography (HPLC); ML-1035, a metoclopramide analogue; gastroprokinetic agent.

INTRODUCTION

ML-1035, 4-amino-5-chloro-2-[2-(methylsulfinyl)-ethoxy]-*N*-[2-(diethylamino)ethyl]benzamide, is a $5HT_3$ receptor antagonist and a new selective gut motility stimulant. ML-1035 is chemically related to metoclopramide, which has been in wide clinical use as an antiemetic and as a stimulant of upper gut motility for more than a decade (1–3). ML-1035 is a sulfoxide compound (Fig. 1). Substantial oxidation to the sulfone and reduction to the sulfide metabolite have been documented after ML-1035 was given to animals (4), and these reactions are similar to the metabolic reactions reported for sulindac (5,6) and sulfinpyrazone (7,8). In addition to the sulfone and the sulfide, *N*-desethyl metabolites were also found to be present after administration of ML-1035 to animals.

To facilitate pharmacokinetic and toxicological studies, we have modified and extended a previously reported automated column-switching HPLC method, which was designed to detect only ML-1035 and its sulfone and sulfide metabolites in rat urine (4), to cover ML-1035 and all of its unconjugated metabolites in plasma. In this HPLC procedure, plasma samples were directly injected onto a short extraction column for sample cleanup and extraction followed by elution onto an analytical column for further analysis, under the control of a column-switching device.

Column-switching HPLC associated with direct injection of biological fluids onto a short extraction column has gained much attention since the first technique was reported a decade ago (9). Endogenous proteins and tenacious lipid components are the two key detrimental factors that one wants to avoid in the development of column-switching HPLC involving direct injection of plasma samples. The present investigation adopted the concept of micellar cleanup using an anionic detergent, sodium dodecyl sulfate (SDS), to solubilize and eliminate the proteins from the extraction column (10,11), while the potential harmful effect of lipids was prevented by periodically rinsing the extraction column with organic solvents after the extracted compounds were switched to the analytical column. In addition to high sensitivity, reproducibility, and sample throughput, the present column-switching HPLC method provides longevity for both extraction and analytical columns. The method has been validated under stringent criteria and routinely applied to various pharmacokinetic studies.

MATERIALS AND METHODS

Chemicals and Reagents

HPLC-grade acetonitrile and isopropyl alcohol were obtained from Burdick and Jackson (Muskegon, MI). Sodium dodecyl sulfate (SDS) was obtained from Sigma Co. (St. Louis, MO). Triethylamine was from Aldrich Chemical Co. (Milwaukee, WI). Tetrahydrofuran was from J. T. Baker (Phillipsburg, NJ). Sodium phosphate, monobasic, was from Mallinckrodt Co. (Paris, KY). ML-1035 sulfoxide, the sulfone, the sulfide, and their respective *N*-desethyl metabolites (Fig. 1) were from Marion Merrell Dow Research Institute (Kansas City, MO). Metoclopramide, used as the internal standard, was obtained from Sigma Co. Acrodisc filter (0.2 μm, product number 4454) was purchased from Gelman Sciences (Ann Arbor, MI).

Apparatus

The HPLC system was from Waters (Milford, MA). The configuration of the column-switching operation is presented in Fig. 2. Pump A (Waters Model 600 Multisolvent Delivery System) and Pump B (Waters Model 510) were used to deliver four different mobile phases. The Waters Autosampler (Model 700 Satellite WISP) was used to inject plasma samples. An electrically driven six-port Autochrom switching

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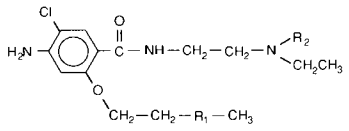

R_1	R_2	COMPOUNDS
SO	CH_2CH_3	sulfoxide
SO_2	CH_2CH_3	sulfone
S	CH_2CH_3	sulfide
SO	H	Desethyl sulfoxide
SO_2	H	Desethyl sulfone
S	H	Desethyl sulfide

Fig. 1. Chemical structures of ML-1035 (SO) and its five metabolites (des SO, SO_2, des SO_2, S, and des S).

valve (Ann Arbor, MI) was used to route the compounds captured on the extraction column to the analytical column. The automatic sample injection, the valve switching, and the extraction column purging events were controlled by the Waters 600E System Controller in connection with the NEC Power Mate-2 Computer (Waters; Milford, MA). Detection was by means of an LS-40 fluorescence detector (Perkin-Elmer, Norwalk, CT) at excitation 308 nm and emission 350 nm.

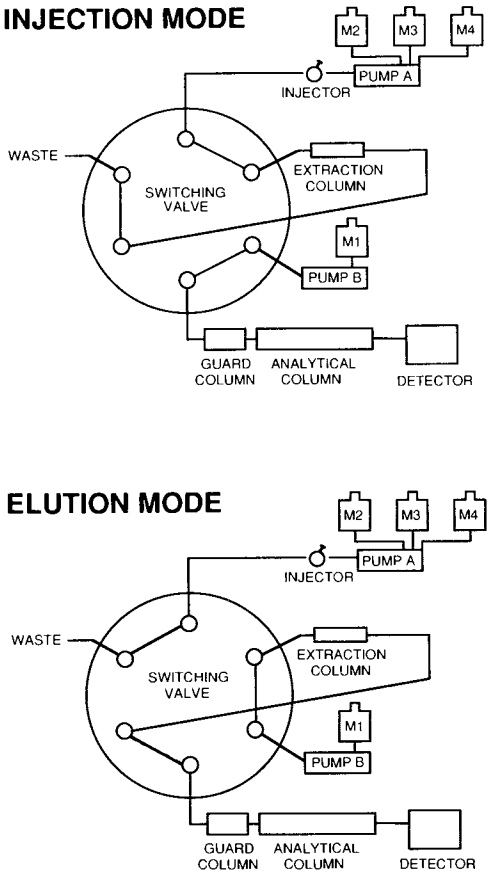

Fig. 2. Schematic representation of a working cycle (injection and elution modes) in the HPLC system involving automatic sample extraction (solid phase) and column switching. The programmable operations and the associated time events are detailed under Materials and Methods. M1 through M4 are four different mobile phases.

Columns and Mobile Phases

The HPLC column system consisted of an extraction and an analytical column. The extraction column was a Resolve CN precolumn (4 × 4.6 mm, 5 μm) from Waters (Milford, MA), which was used to retain ML-1035 and the related compounds until elution. The extraction column is short and inexpensive and incorporates a radial distribution device that spreads the injected sample over the frit surface. This allows the retained compounds to adhere to a narrow band and also reduces the likelihood the frits will become clogged after a number of plasma injections (12,13). A Spherisorb C_8 column (150 × 4.6 mm, 5 μm) (Alltech Associates; Deerfield, IL) was used to separate the drug compounds after elution from the extraction column. The analytical C_8 column was guarded by a Waters C_8 precolumn (4 × 4.6 mm, 5 μm). Four different mobile phases were used: M1, 32.5% acetonitrile in 50 m*M* NaH_2PO_4 containing 0.25% SDS and 0.2% triethylamine (pH 6.1); M2, 0.5% SDS in 50 m*M* NaH_2PO_4 (pH 4.3); M3, 0.25% SDS and 2% acetonitrile in 50 m*M* NaH_2PO_4 (pH 4.3); and M4, acetonitrile–isopropanol–tetrahydrofuran (69.9:30:0.1, v/v). M2, M3, and M4 were washing solvents delivered by Pump A, while M1 was elution buffer delivered by Pump B (Fig. 2). All mobile phases were degassed with helium prior to use.

Sample Processing

Stock solutions of 1.0 mg/ml were prepared in methanol for ML-1035 sulfoxide, sulfone, sulfide, their respective *N*-desethyl metabolites, and the internal standard (metoclopramide). Stock solutions of ML-1035 and its metabolites were combined and diluted with methanol to 0.1 mg/ml. They were stored at 5°C. Further dilutions to 0.01, 0.001, and 0.0001 mg/ml were made in 50 m*M* sodium phosphate, monobasic, pH at 4.3. Plasma standards containing ML-1035 and its five metabolites were prepared by spiking pooled drug-free dog or rat plasma (250 μl for each) at concentrations of 10, 15, 20, 50, 100, 200, 500, 1000, and 1500 ng/ml. The plasma standards and the unknown plasma samples were spiked with 50 μl of 0.001 mg/ml internal standard in 50 m*M* phosphate. These samples were then diluted to 500 μl with 50 m*M* phosphate and passed through the Acrodisc filter (0.2 μm) prior to injection of 200 μl of the resulting mixture into the HPLC.

Cycle of Automated Analytical Procedure

A schematic of the column-switching HPLC system has been illustrated in Fig. 2. All experiments were conducted at room temperature. A complete automated plasma sample analysis lasted 30 min including the following programmable events, which were controlled by the NEC Power Mate-2 computer.

Step A (0–3.0 min, Sample Extraction and Cleanup). An aliquot of 200 μl diluted (1:1) plasma sample was injected onto the extraction column for sample cleanup and extraction (injection mode). Proteins and other polar components were solubilized and flushed to waste with M2 containing 0.5% SDS at 1 ml/min, while lipophilic compounds including ML-1035 and its metabolites were retained on the extraction

column. SDS is an anionic detergent which, at 0.5% (17.3 m*M*), served as a key component in the micellar cleanup process.

Step B (3.0–5.0 min, Sample Elution). At 3.0 min the switching valve was automatically rotated to the elution mode. The retained drug compounds were then eluted with M1 containing 0.25% SDS at 2 ml/min from the extraction column onto the analytical column for further separation and quantitation. Note that this is a forward flush pattern. The Waters Guard-Pak precolumn is not compatible with back flush.

Step C (5.0–7.0 min, Extraction Column Cleanup). At 5.0 min the switching valve was rotated back to the injection mode. The extraction column was then purged again with M2 containing 0.5% SDS at 2 ml/min. This step was performed to clean up the potential residual denatured proteins which might stick to the extraction column packing due to the strong mobile phase M1 containing 32.5% ACN.

Step D (7.0–10.0 min, Extraction Column Cleanup). The extraction column was purged with M3 containing 0.25% SDS and 2% ACN at 2 ml/min. In addition to purging, M3 served as a buffer for the smooth transition from M2 to M4.

Step E (10.0–13.0 min, Extraction Column Cleanup). The extraction column was then purged with the organic solvent mixture M4 at 2 ml/min to remove any endogenous hydrophobic components that might have been retained on the extraction column.

Step F (13.0–15.0 min, Extraction Column Cleanup). The extraction column was purged with M3 containing 0.25% SDS and 2% ACN at 1.0 ml/min. M3 was used here as a buffer for the smooth transition from M4 to M2.

Step G (15.0–30.0 min, Extraction Column Reconditioning). The extraction column was reequilibrated with M2 containing 0.5% SDS at 0.5 ml/min. At the end of Step G the chromatographic data were generated by the computing integrator and the HPLC system was ready for the next injection.

All steps, except Step E, involved solvents containing SDS to remove proteinaceous components. Step E was designed to eliminate the potential endogenous lipids from the extraction column using an organic solvent mixture. Step E also helps remove hydrophobic contaminants, if any, in the detergent SDS. These purging events contributed significantly to the preservation of both extraction and analytical columns.

Calibration, Calculation, and Application

For each assay, nine plasma standards in a concentration range of 10 to 1500 ng/ml were prepared in duplicate. The in-house CALS system was used to collect, integrate, and analyze all data. All quantitations of drug and metabolite concentrations were based on the peak height ratio except for the extraction efficiency, for which the peak area was used. The calibration curve for each individual compound was obtained by the least-squares linear regression of the peak height ratios versus concentrations. These curves were then used to interpolate the concentrations of the parent drug and the metabolites in plasma samples. The present column-switching HPLC method has been extensively applied to pharmacokinetic studies in animals receiving intravenous and oral doses of ML-1035.

RESULTS AND DISCUSSION

Sample Pretreatments and Extraction Efficiency

The present automated column-switching HPLC assay requires minimum sample pretreatments: centrifugation to obtain particulate-free plasma samples, dilution with phosphate buffer to increase stability and reduce viscosity of the samples, and filtration to eliminate hidden particles and bacteria. These pretreatment steps were important for good column performance. Filtration through a 0.2-μm filter to render the plasma samples sterile and particulate-free did not cause significant loss of the tested compounds spiked in the plasma, as the peak heights were not different between the filtered and the unfiltered samples (Table I).

The absolute extraction efficiency of the extraction column was first evaluated by comparing the results from the column-switching method to those from the direct injection of the buffered solutions (50 m*M* phosphate, pH 4.3) onto the analytical column. The mean recoveries for ML-1035 (SO), the sulfone (SO_2), the sulfide (S), *N*-desethyl ML-1035 (Des SO), *N*-desethyl sulfone (Des SO_2), and *N*-desethyl sulfide (Des S) were 86.0, 85.0, 84.1, 87.5, 86.0, and 85.4%, respectively (Table II). The relative extraction efficiency was also evaluated by comparing the peak areas for the tested compounds in plasma to those in phosphate buffer under the same column-switching condition. Peak areas for the compounds in dog plasma at three concentrations (100, 500, and

Table I. Peak Heights of ML-1035, Its Five Metabolites, and the Internal Standard (Metoclopramide) in Filtered (F) and Unfiltered (U) Plasma with 0.2-μm Acrodisc Filter; Mean (SD), $N = 3$

Compound spiked[a]	Dog plasma		Human plasma	
	F	U	F	U
ML-1035	83.6 (0.5)	82.5 (0.4)	84.0 (0.4)	85.9 (0.5)
Des ML-1035	108.1 (0.6)	105.9 (0.7)	105.3 (4.3)	105.9 (2.0)
Sulfone	55.0 (0.4)	54.5 (0.4)	55.1 (0.4)	55.3 (0.4)
Des sulfone	50.9 (0.3)	50.5 (0.3)	50.5 (1.4)	51.0 (0.4)
Sulfide	30.7 (0.4)	31.2 (0.4)	29.8 (0.4)	30.4 (0.3)
Des sulfide	34.8 (0.4)	34.7 (0.2)	34.8 (0.3)	35.1 (0.5)
Metoclopramide	70.0 (0.3)	69.9 (0.3)	70.3 (0.3)	71.8 (1.0)

[a] Spiked concentrations at 100 ng/ml for all compounds except for metoclopramide (250 ng/ml).

Table II. Extraction Efficiency of the Extraction Column[a]

Concentration (ng/ml)	ML-1035	Sulfone	Sulfide	Des ML-1035	Des sulfone	Des sulfide
100	86.9	86.0	85.8	88.1	86.6	85.1
500	85.4	84.7	82.6	87.9	85.8	85.3
1000	85.7	84.3	83.9	86.5	85.6	85.8
Mean ± SD	86.0 ± 0.8	85.0 ± 0.9	84.1 ± 1.6	87.5 ± 0.9	86.0 ± 0.5	85.4 ± 0.4

[a] The percentage extraction was the ratio of the peak area of each tested compound in dog plasma under column-switching conditions to that in phosphate buffer under conditions of direct injection onto the analytical column.

1000 ng/ml) did not differ significantly from those in phosphate buffer (data not shown), indicating that plasma factors including protein binding of the compounds did not hamper the performance of the extraction column.

Specificity, Linearity, and Sensitivity

SO, SO_2, S, Des SO, Des SO_2, Des S, and the internal standard (IS) in dog (Fig. 3) and rat (Fig. 4) plasma were well separated under the experimental conditions. Chromatograms shown in panels A and B of Figs. 3 and 4 indicate that there were no interfering peaks from the plasma constituents of the two different animal species. The peak height ratios were linearly related to concentrations over the ranges of 10 to 100 and 100 to 1500 ng/ml for SO, SO_2, Des SO, and Des SO_2 in dog and rat plasma. Good linearity for Des S and S was found to be over the ranges of 15 to 200 and 100 to 1500 ng/ml. Correlation coefficients were greater than 0.999 for all the compounds tested. The lowest concentration that could be precisely and accurately quantitated was at least 10 ng/ml for SO, SO_2, Des SO, and Des SO_2 and 15 ng/ml for Des S and S using an injection volume of 200 μl after 1:1 dilution with 50 m*M* phosphate (Table IV). At the limit of quantitation, the signal-to-noise ratio on the chromatogram was greater than 10 for all the compounds. The quantitation limit

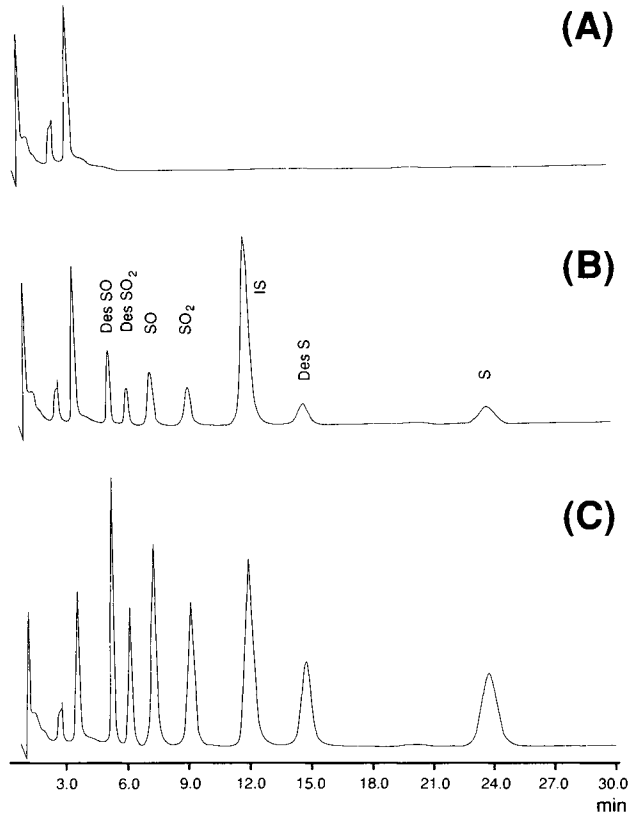

Fig. 3. Typical representative chromatograms from dog plasma. (A) Blank plasma; (B) plasma spiked with ML-1035 (SO), sulfone (SO_2), sulfide (S), *N*-desethyl ML-1035 (des SO), *N*-desethyl sulfone (des SO_2), and *N*-desethyl sulfide (des S) at 50 ng/ml and the internal standard (IS) at 250 ng/ml; (C) plasma spiked with the same compounds but at 200 ng/ml for SO and its derivatives.

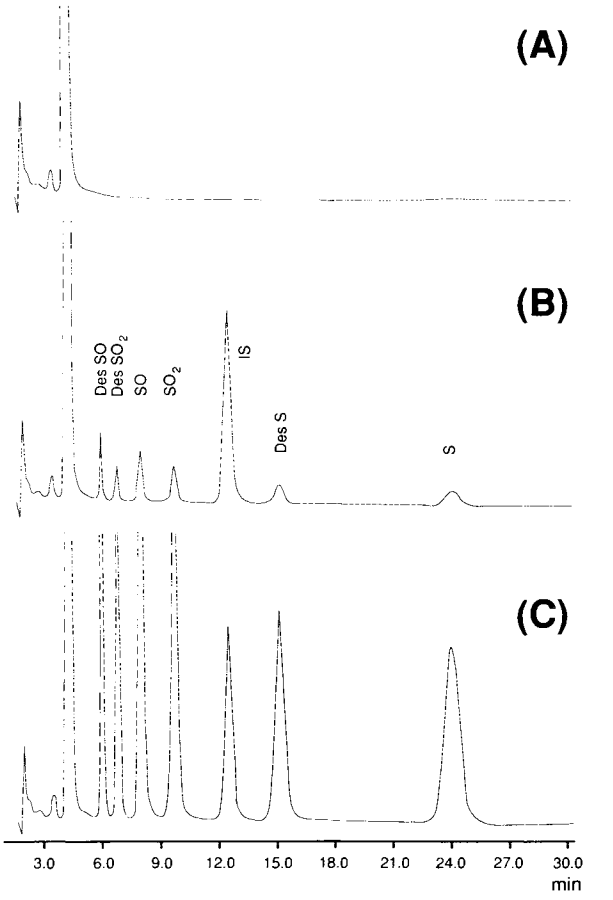

Fig. 4. Typical representative chromatograms from rat plasma. (A) Blank plasma; (B) plasma spiked with ML-1035 (SO), sulfone (SO_2), sulfide (S), *N*-desethyl ML-1035 (des SO), *N*-desethyl sulfone (des SO_2), and *N*-desethyl sulfide (des S) at 50 ng/ml and the internal standard (IS) at 250 ng/ml; (C) plasma spiked with the same compounds but at 500 ng/ml for SO and its derivatives.

Table III. Within-Run Precision (% CV) and Accuracy (% Error) of ML-1035 and Its Five Metabolites in Dog Plasma

Predicted conc. (ng/ml)	Observed conc. (ng/ml), mean (% CV)(% error); $N = 4$					
	ML-1035	Des ML-1035	Sulfone	Des sulfone	Sulfide	Des sulfide
20	20.2 (1.1)(1.2)	20.1 (1.9)(0.6)	20.1 (4.0)(0.7)	20.2 (3.2)(1.0)	20.3 (2.2)(1.5)	19.4 (10.7)(2.9)
100	99.8 (0.7)(0.2)	99.6 (0.7)(0.4)	99.8 (1.1)(0.2)	100.1 (0.7)(0.1)	96.1 (0.8)(3.9)	97.7 (1.9)(2.3)
500	518.2 (6.4)(3.6)	521.1 (6.4)(4.2)	516.3 (6.5)(3.3)	517.8 (6.4)(3.6)	510.2 (6.7)(2.0)	511.2 (6.6)(2.2)
1500	1504.0 (0.8)(0.3)	1508.0 (1.2)(0.5)	1504.0 (0.9)(0.3)	1503.0 (0.8)(0.2)	1508.0 (1.1)(0.5)	1506.0 (0.7)(0.4)

can be further lowered by injecting up to 1 ml of the sample mixture onto the extraction column.

Precision and Accuracy

The within-run precision and accuracy of the method were examined at three concentrations in dog and rat plasma after calculating observed concentrations from the actual response values (ratios) and the regression curves. For dog plasma spiked with the tested compounds over the range of 20 to 1500 ng/ml (Table III), the precision of the assay was excellent, with coefficients of variation of the calculated observed concentrations being in the range of 0.7 to 10.7%. Compared to the predicted concentrations, the observed concentrations of the tested compounds also reflected good accuracy of the method, with relative errors falling in the range of 0.1 to 4.2%. The between-run precision and accuracy of the method were investigated on three occasions using a concentration range of 10 to 1500 ng/ml. Using dog plasma as an example, the day-to-day precision was excellent, with coefficients of variation being in the range of 0.8 to 9.6% for all the compounds tested (Table IV). Compared to the predicted concentrations, the observed concentrations of the tested compounds also reflected the good accuracy of the method, with relative errors falling in the range of 0.1 to 3.9%. Results of the within- and the between-run precision and accuracy were similar for rat plasma (data not shown). These experimental results demonstrate that the present HPLC method is highly reproducible.

Stability of Plasma Sample and Column Performance

Stability of the plasma samples after dilution with buffer was first evaluated by 64 consecutive injections of the same dog plasma sample over 38 hr at room temperature. As can be seen in Fig. 5, there were no significant changes in peak height ratio between the first four and the last four injections for all tested compounds. Unaltered peak height ratio also suggests that the performance of the single short extraction column remains excellent throughout a number of injections of the plasma samples in terms of peak shape, peak size, and retention time. Absolute peak responses of analytes and internal standard were not significantly changed after a number of injections (Table I). Figure 6 demonstrates excellent column performance as the chromatogram of the 64th injection is almost identical to that of the first injection. The same extraction column was loaded with additional 100 samples after the 64th injection and the column performance was still found to be excellent. These results suggest that the extraction column could endure 164 injections or more (about 16 ml plasma or more) without buildup of column pressure. The resulting chromatograms as shown in Fig. 6 also suggest that the drug and its metabolites did not decompose during the test period (38 hr) at room temperature. Once an extraction column has been selected for further testing, challenging the extraction column by consecutive injections of the same plasma sample would probably be the most crucial event in method validation. Column-switching HPLC, which involves direct injection of biological fluids onto the extraction column, would offer no significant advantage over the con-

Table IV. Between-Run Precision (% CV) and Accuracy (% Error) of ML-1035 and Its Five Metabolites in Dog Plasma on Three Different Occasions ($N = 6$) During the Validation Process[a]

Predicted conc. (ng/ml)	Observed concentration (ng/ml), mean (% CV) (% error)					
	ML-1035	Des ML-1035	Sulfone	Des sulfone	Sulfide	Des sulfide
10	10.0 (1.2)(0.3)	9.7 (5.4)(2.6)	10.1 (3.0)(0.5)	10.1 (3.7)(0.5)	—	—
15	15.3 (5.5)(2.0)	15.0 (7.3)(0.1)	15.1 (5.6)(0.3)	15.3 (6.0)(1.7)	14.9 (8.4)(0.7)	14.8 (9.6)(1.5)
20	20.2 (1.8)(1.2)	19.8 (3.4)(1.0)	20.0 (2.0)(0.2)	19.9 (3.5)(0.4)	19.1 (6.7)(4.4)	19.7 (7.4)(1.7)
50	49.3 (3.4)(1.4)	49.4 (4.1)(1.2)	49.5 (3.0)(1.0)	49.6 (2.0)(0.9)	48.7 (3.0)(2.6)	49.4 (4.4)(1.2)
100	100.3 (1.5)(0.3)	100.4 (1.7)(0.4)	100.2 (1.8)(0.2)	100.2 (1.5)(0.2)	100.4 (4.4)(0.4)	96.1 (3.8)(3.9)
200	198.1 (1.6)(1.0)	199.2 (2.5)(0.4)	197.6 (1.9)(1.2)	198.2 (2.1)(0.9)	200.2 (1.9)(0.1)	199.5 (1.5)(0.3)
500	504.3 (2.8)(0.9)	507.7 (3.0)(1.5)	500.5 (2.6)(0.1)	502.8 (3.5)(0.6)	493.4 (2.3)(1.3)	495.4 (2.7)(0.9)
1000	994.7 (2.6)(0.5)	994.9 (2.3)(0.5)	997.3 (2.8)(0.3)	994.4 (2.6)(0.6)	996.5 (3.2)(0.4)	995.5 (2.7)(0.5)
1500	1502.0 (1.2)(0.1)	1501.0 (0.8)(0.1)	1502.0 (1.6)(0.1)	1503.0 (1.4)(0.2)	1505.0 (1.6)(0.3)	1505.0 (1.4)(0.3)

[a] Duplicate experiments were run for each occasion on 3 different days.

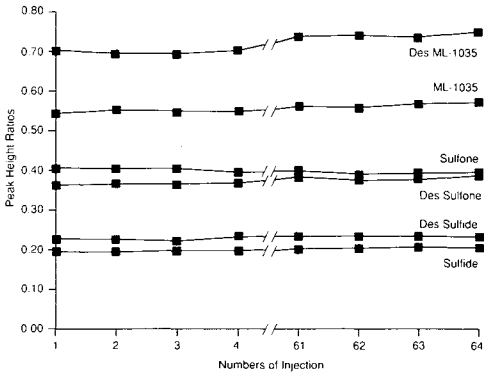

Fig. 5. Peak height ratios of the tested compounds (100 ng/ml for each) obtained from the first four and the last four of the same dog plasma sample in 64 consecutive injections over a period of about 38 hr.

ventional HPLC if the extraction column could not endure a number of injections.

Successful development of column-switching HPLC is also dependent on the selection of purging solvents to clean up the plasma sample and the extraction column. It is very likely that the use of sodium dodecyl sulfate (SDS) to eliminate proteins by solubilization and the use of strong organic solvents to purge the extraction column periodically to remove lipids in the present study have contributed to the excellent longevity of the extraction column. It has been long known that SDS, an anionic detergent, solubilizes proteins (14) in the micelles it forms. The application of SDS or other detergents to column-switching HPLC has gained increasing attention (10,11,15–20), although it has been used in micellar chromatography associated with direct injection of biological fluids (21–23). One has to be aware that, in addition to proteinaceous components, the endogenous hydrophobic components in biological fluids are equally detrimental to the extraction column. This problem could be corrected by rinsing the extraction column with strong organic solvents such as mobile phase M4. For excellent column performance, therefore, purging the extraction column between each individual analysis seems to be indispensable. Under the present experimental conditions, and with replacement of a new extraction column every 100 to 200 injections, the analytical column had a life span of more than 5000 plasma injections with excellent performance. Achievement of such a high longevity for analytical column may be due in part to the use of SDS in the separation buffer M1. Filtration through a 0.2-μm filter and application of Waters Guard-Pak precolumn for sample extraction also contributed to the success of the present high-performance column-switching chromatography.

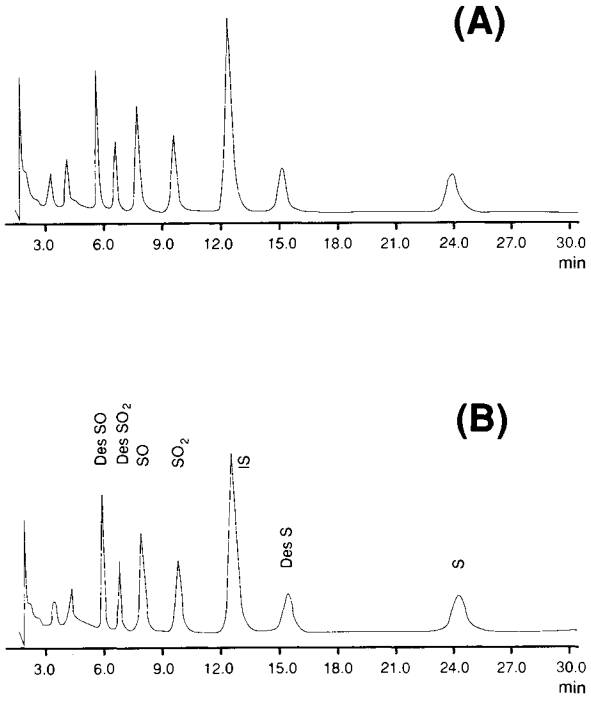

Fig. 6. Two representative chromatograms showing the reproducible results of the same plasma sample at room temperature after 64 consecutive injections, which covered a period of about 38 hr. A and B are the corresponding chromatograms of the 1st and the 64th injection, respectively, as shown in Fig. 5. The samples were spiked with ML-1035 (SO), sulfone (SO_2), sulfide (S), *N*-desethyl ML-1035 (des SO), *N*-desethyl sulfone (des SO_2), and *N*-desethyl sulfide (des S) at 100 ng/ml and the internal standard (IS) at 250 ng/ml. Note that A and B are in the same peak response scale in millivolts (mV).

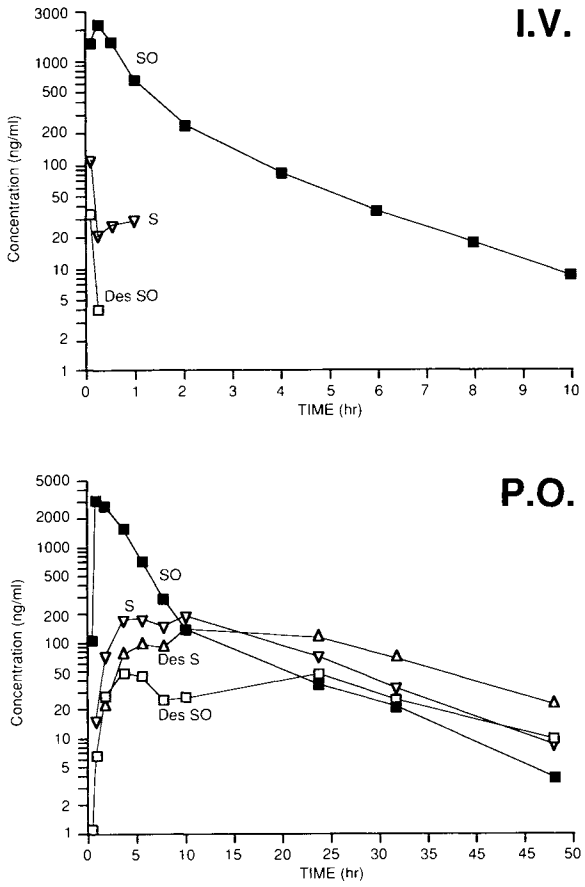

Fig. 7. Plasma disposition profiles of ML-1035 (SO) and its metabolites (des SO, S, and des S) from a dog receiving an i.v. (5 mg/kg) and a p.o. dose (40 mg/kg) of SO on two separate occasions.

In Vivo Application

The present HPLC method has been routinely applied to analyze dog and rat plasma samples obtained from the pharmacokinetic studies. The method is also applicable to the analysis of human plasma samples. Shown in Fig. 7 are plasma disposition profiles after intravenous (5 mg/kg) and oral (40 mg/kg) dosing to the same dog in a crossover study. The preliminary results suggest the sulfide is the major metabolite and that more metabolites are generated after oral administration. Results from the dog study also demonstrate that the present method is a simple, practical, and reproducible method for quantitation of ML-1035 and its derivatives in plasma with a high sample throughput. The simplicity of the present HPLC method, which employed only one switching valve, two pumps, and micellar cleanup in direct plasma injection, has made the column-switching technique coupled with micellar cleanup a very useful analytical tool in routine assays of drug and metabolites in biological fluids. In addition, the present state-of-the-art HPLC procedure can be adapted to assay of any of metoclopramide analogues in full automation without resorting to the use of robots.

REFERENCES

1. G. I. Sanger and F. D. King. From metoclopramide to selective gut motility stimulants and 5-HT_3 receptor antagonists. *Drug Design Deliv.* 3:273–295 (1988).
2. R. M. Pinder, R. N. Brogden, P. R. Sawyer, T. M. Speight, and G. S. Avery. Metoclopramide. A review of its pharmacological properties and clinical use. *Drugs* 12:81–131 (1976).
3. R. A. Harrington, C. W. Hamilton, R. N. Brogden, J. A. Linkewick, J. A. Romankiewicz, and R. C. Heel. Metoclopramide. An updated review of its pharmacological properties and clinical use. *Drugs* 25:451–494 (1983).
4. B.-S. Kuo, J. C. Poole, A. Mandagere, and K.-K. Hwang. Column-switching HPLC determination of ML-1035 sulfoxide and its sulfone and sulfide metabolites in rat urine. *J. Pharm. Biomed. Anal.* (In press) (1992).
5. D. E. Duggan, K. F. Hooke, R. M. Noll, H. B. Hucker, and C. G. Van Arman. Comparative disposition of sulindac and metabolites in five species. *Biochem. Pharmacol.* 27:2311–2320 (1978).
6. J. H. Ratnayake, P. E. Hanna, M. W. Anders, and D. E. Duggan. Sulfoxide reaction. In vitro reduction of sulindac by rat hepatic cytosolic enzymes. *Drug Metab. Dispos.* 9:85–87 (1981).
7. B.-S. Kuo and W. A. Ritschel. Pharmacokinetics and reversible biotransformation of sulfinpyrazone and its metabolites in rabbits. I. Single-dose study. *Pharm. Res.* 3:173–177 (1986).
8. B.-S. Kuo and W. A. Ritschel. Pharmacokinetics and reversible biotransformation of sulfinpyrazone and its metabolites in rabbits. II. Multiple-dose study. *Pharm. Res.* 3:178–183 (1986).
9. W. Roth, K. Beschke, R. Jauch, A. Zimmer, and F. W. Koss. Fully automated high-performance liquid chromatography. A new chromatograph for pharmacokinetic drug monitoring by direct injection of body fluids. *J. Chromatogr.* 222:13–22 (1981).
10. G. Tamai, H. Yoshida, and H. Imai. High-performance liquid chromatographic drug analysis by direct injection of whole blood samples. I. Determination of moderately hydrophobic drugs incorporated into blood corpuscles. *J. Chromatogr.* 423: 147–153 (1987).
11. J. V. Posluszny and R. Weinberger. Determination of drug substances in biological fluids by direct injection multidimensional liquid chromatography with a micellar cleanup and reversed-phase chromatography. *Anal. Chem.* 60:1953–1958 (1988).
12. J. W. Veals and C. Lin. Column-switching HPLC methods for drug analysis in biological fluids. *Am. Lab.* 20:42–47 (1988).
13. B.-S. Kuo, A. Mandagere, D. R. Osborne, and K.-K. Hwang. Column-switching high-performance liquid chromatographic (HPLC) determination of hydrochlorothiazide in rat, dog, and human plasma. *Pharm. Res.* 7:1257–1261 (1990).
14. K. Weber and M. Osborn. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:44–49 (1969).
15. G. Tamai, H. Yoshida, and H. Imai. High-performance liquid chromatographic drug analysis by direct injection of whole blood samples. II. Determination of hydrophilic drugs. *J. Chromatogr.* 423:155–161 (1987).
16. G. Tamai, H. Yoshida, and H. Imai. High-performance liquid chromatographic drug analysis by direct injection whole blood samples. III. Determination of hydrophobic drugs absorbed on blood cell membranes. *J. Chromatogr.* 423:163–168 (1987).
17. G. Tamai and H. Imai. Determination of propanolol and 4-hydroxypropanolol in tissue homogenates by direct injection and column-switching HPLC. *Chem. Pharm. Bull.* 38:810–811 (1990).
18. M. J. Koenigbauer and M. A. Curtis. Use of micellar mobile phases and microbore column switching for the assay of drug in physiological fluids. *J. Chromatogr.* 427:277–285 (1988).
19. J. V. Posluszny, R. Weinberger, and E. Woolf. Optimization of multidimensional high-performance liquid chromatography for the determination of drugs in plasma by direct injection, micellar cleanup and photodiode array detection. *J. Chromatogr.* 507:267–276 (1990).
20. D. Bentrop, F. V. Warren, S. Schmitz, and B. A. Bidling-Meyer. Analysis of carbamazepine in serum by liquid chromatography with direct sample injection and surfactant containing eluents. *J. Chromatogr.* 535:293–304 (1990).
21. M. Arunyanart and L. J. Cline Love. Determination of drugs in untreated body fluids by micellar chromatography with fluorescence detection. *J. Chromatogr.* 342:293–301 (1985).
22. J. G. Dorsey. Micellar liquid chromatography. *Adv. Chromatogr.* 27:167–214 (1987).
23. K. B. Sentell, J. F. Clos, and J. G. Dorsey. A practical approach to direct injection of untreated physiological fluids with micellar liquid chromatography: Determination of bumetanide. *BioChromatogr.* 4:35–40 (1989).