Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 1250–1255

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Effective on-line extraction of drugs from plasma using a restricted-access media column in column-switching HPLC equipped with a dilution system: Application to the simultaneous determination of ER-118585 and its metabolites in canine plasma

Eiichi Yamamoto [a,*], Takashi Kato [a], Nariyasu Mano [b], Naoki Asakawa [c]

[a] *Analytical Research Laboratories, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan*
[b] *Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan*
[c] *Drug Development Technology, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan*

ARTICLE INFO

Article history:
Received 22 December 2008
Received in revised form 17 February 2009
Accepted 20 February 2009
Available online 4 March 2009

Keywords:
Direct injection
Restricted-access media
On-line extraction
On-line dilution
Protein binding

ABSTRACT

The present paper describes the on-line extraction of drugs in plasma using a restricted-access media (RAM) column in a column-switching high performance liquid chromatography (HPLC) apparatus that was equipped with an on-line dilution system. The use of a six- to eightfold on-line dilution ratio for plasma samples resulted in almost 100% recovery of both acidic and basic drugs from plasma. It was found that the relationship between the on-line dilution times and drug recovery efficiencies from plasma was explained in terms of the binding constant between the drug and albumin. The applicability of column-switching HPLC with an on-line dilution system and the effectiveness of the extraction procedure were confirmed by a simultaneous determination of the basic compound, ER-118585, and its metabolites in canine plasma.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The direct analysis of drugs and metabolites in biological fluids such as plasma using restricted-access media (RAM) [1,2] with column-switching high performance liquid chromatography (HPLC) can rapidly yield sensitive chemical identification results. Today's commercially available RAM columns are sufficiently stable to allow for their routine use in the pharmaceutical industry [3,4], where they help to automate the on-line preparation of drug samples in biological fluids [2,5]. An additional advantage of this methodology is that it allows for a reduction of the matrix effect in drug analysis in plasma using HPLC–electrospray ionization-mass spectrometry (ESI-MS) [6]. Direct injection of the plasma sample offers simple, reliable, and automated sample preparation with a relatively simple apparatus. The strategy is attractive for clinical drug analysis. It is valuable in therapeutic drug monitoring (TDM), which requires prompt determination of the plasma levels of administered drugs in order to adjust the dosage on a patient-by-patient basis.

However, although a RAM column was invented 20 years ago [7], only a limited amount of research has explored the quantitative analysis of drugs and metabolites by direct injection [2,3,8–13]. Moreover, only a handful of reports have described the direct analysis of acidic drugs [14–17].

Silica-based ion-exchange RAM columns [18–22] can perform selective extraction of acidic and basic drugs from plasma in comparison with the reversed-phase RAM column. However, even with ion-exchange RAM columns, it is challenging to efficiently extract acidic drugs. Drugs in plasma generally exist in two forms: bound and unbound. When a plasma sample-containing drug is injected onto a RAM column, initially only the unbound drug penetrates and is retained on the stationary phase of the RAM material. Thereafter, the bound drug, which is released from the protein consistent with the drug–protein binding equilibrium in the mobile phase, penetrates the stationary phase. This implies that in the direct analysis of drugs using RAM columns, the analytes must be released from their binding proteins during the extraction process.

Acidic and neutral drugs will primarily bind to albumin, which is the most abundant plasma protein. To extract acidic drugs from plasma, it is necessary to take into account the strong protein binding of acidic drugs. In any drug–protein binding, hydrophobic interactions play an important role. Accordingly, the addition of a small amount ($\leq$10%) of organic modifier to the extraction

* Corresponding author.
E-mail address: e-yamamoto@hhc.eisai.co.jp (E. Yamamoto).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.02.022

mobile phase enhances the release of the drug from the binding protein [23]. This is an effective procedure to improve the extraction efficacy of drugs from plasma. However, there is stillroom for improvement. The extraction of drugs with RAM columns is often associated with insufficient drug recovery rates due to protein binding.

To improve drug recovery rates in the context of on-line extraction following direct plasma injection, a column-switching HPLC methodology that features an on-line dilution system was developed [24].

This report describes a column-switching HPLC that enables the effective and simple on-line extraction of ketoprofen as model acidic drug and propranolol and imipramine as basic drugs using a methylcellulose-immobilized-octadecylsilane (MC-ODS) column, which is an example of a RAM solid-phase extraction (SPE) column. In order to confirm the applicability of the column-switching HPLC and the extraction procedure, simultaneous determination of ER-118585, an erectile dysfunction agent based on selective phosphodiesterase-5 inhibition [25], and its metabolites including a carboxylic acid metabolite in canine (beagle) plasma was conducted.

2. Experimental

2.1. Chemicals and reagents

Ketoprofen, ammonium acetate and acetonitrile were purchased from Wako Pure Chemicals (Osaka, Japan). Propranolol and imipramine were purchased from Sigma–Aldrich (St. Louis, MO, USA). All reagents were of analytical grade and used without further purification. Water was deionized and purified by a Milli-Q® TOC purification system from Millipore (Bedford, MA, USA). ER-118585 and its four metabolites: ER-120017 (ketone metabolite), ER-120018 (desmethyl metabolite), ER-120435 (lactone metabolite) and ER-132331 (carboxylate metabolite) were synthesized by Eisai Co., Ltd. (Tokyo, Japan).

2.2. Sample preparation

Following anti-coagulation with heparin, human plasma and beagle dog plasma were obtained by centrifugation of healthy human blood and beagle blood, respectively. The centrifuged plasma was used as the “blank plasma” sample.

Primary stock solutions (100 μg/mL) of ketoprofen, propranolol and imipramine were prepared in 50% acetonitrile. Human plasma containing each drug was prepared by adding the drug to blank plasma at concentrations of 1 μg/mL.

Primary stock solutions (100 μg/mL) of a mixture of ER-118585 and its metabolites were prepared in 30% acetonitrile. Beagle dog plasma containing a mixture of ER-118585 and its metabolites was prepared by adding the compounds to blank plasma at concentrations of 25, 50, 250, 500 and 2500 ng/mL. The calibration standard solutions were prepared in 30% acetonitrile using the same compound concentrations as for the drug-supplemented plasma samples.

2.3. Instrumentation

Two systems were used for column-switching HPLC. The first was an extraction LC that was equipped with a bypass line, which was equipped with a pump for on-line extraction [24]. The second was a dual pump dilution apparatus, as illustrated in Fig. 1. This system consisted of two LC-20AD pumps for the extraction LC, an LC-20AB (dual pump model) for the analytical LC, a SIL-20AC auto-injector, a DGU-20 degasser, a CTO-20A column oven, an FCV-20AH six-port valve, an SPD-20A UV detector, and a CBM-20A communication bass module. The LC-solution software package was used for liquid chromatography controls, data acquisition, and data analysis. A MC-ODS SPE column (10 mm × 4.0 mm I.D., 50-μm particle, 12-nm pore size) was used as the extraction column, together with a YMC Pro-C18 (150 mm × 4.6 mm I.D., 5-μm particle, 12-nm pore size) analytical column (Kyoto, Japan) in Sections 2.4 and 2.5, and a GL-science Inertsil ODS-2 (Tokyo, Japan) analytical column (150 mm × 4.6 mm I.D., 5-μm particle, 12-nm pore size) in Sections 2.6 and 2.8.

2.4. Effect of the off-line dilution of the plasma sample on the recovery of ketoprofen

An aliquot of a human plasma sample supplemented with 1 μg/mL of ketoprofen was diluted at ratios of 2× and 10× with phosphate buffered saline (resulting ketoprofen concentrations: 500 and 100 ng/mL).

A 10 μL aliquot of intact human plasma sample, 20 μL of 2× diluted sample, and 100 μL of 10× diluted sample were separately injected onto the MC-ODS SPE column in a standard column-switching HPLC apparatus. The recovery of ketoprofen (100 ng) under each condition was evaluated by comparing the results with those of the standard solution (50% acetonitrile solution).

The injected samples were delivered from the injector to the MC-ODS SPE column with a mixture of 20 mmol/L phosphoric acid and acetonitrile (90:10, v/v) at a flow rate of 2 mL/min. Compounds were concentrated on the SPE column for 2 min in the context of the extraction mobile phase. Following the removal of plasma proteins and other matrix components, the compounds that had been enriched on the SPE column were backflushed to the analytical column by switching a six-port valve into a gradient elution mode at a flow rate of 1.0 mL/min. Analytes were detected using an SPD-10Avp UV detector at a wavelength of 254 nm. The mobile phases consisted of (A) a mixture of water and acetonitrile (90:10, v/v) containing 0.1% trifluoroacetic acid and (B) a mixture of water and acetonitrile (10:90, v/v) containing 0.1% trifluoroacetic acid.

2.5. Effects of extraction flow rate and injection volume on the recovery of drugs from plasma

The effects of the extraction flow rate (0.2, 1.0 and 2.0 mL/min) and injection volume of the human plasma sample (10, 50 and 100 μL containing 100 ng of ketoprofen or propranolol) on drug recovery from plasma was evaluated using the MC-ODS SPE column in a standard column-switching HPLC unit. The extraction mobile phase featured a mixture of 20 mmol/L phosphoric acid (pH 2) – acetonitrile (90:10, v/v) for ketoprofen and 20 mmol/L phosphate buffer (pH 7) – acetonitrile (90:10, v/v) for propranolol. The detection wavelength for propranolol was 280 nm. All other conditions were as described above.

2.6. Effect of the on-line dilution ratio on the recovery of drugs from plasma using the on-line extraction technique

In on-line extraction of drugs from plasma using column-switching HPLC system with dilution system, the injected plasma sample was diluted with extraction mobile phase and delivered to the RAM column. The effect of on-line dilution on the effectiveness of drug recovery from human plasma using the MC-ODS SPE column was assessed. The extraction mobile phase was a mixture of 20 mmol/L phosphoric acid (pH 2) – acetonitrile (90:10, v/v) for ketoprofen and 20 mmol/L phosphate buffer (pH 7) – acetonitrile (90:10, v/v) for propranolol and imipramine. In on-line extraction, 2 min extraction at a flow rate of 2 mL/min, with or without on-line dilution was prepared for the compounds. One hundred microliters

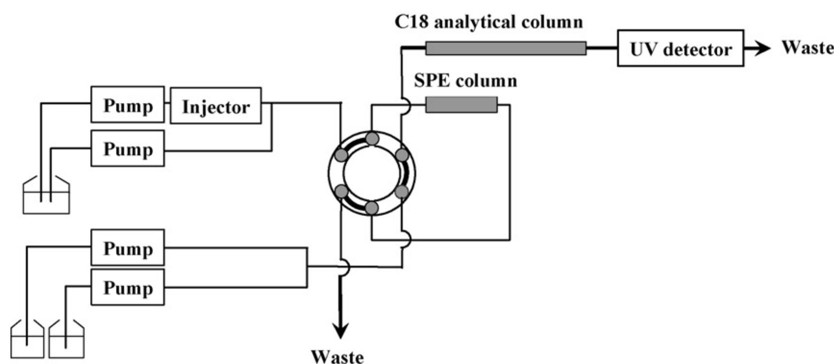

Fig. 1. Schematic of the column-switching HPLC-UV apparatus.

of the plasma sample supplemented with each drug (1 μg/mL) was injected onto the MC-ODS SPE column.

2.7. Simultaneous determination of ER-118585 and its metabolites in beagle dog plasma

To analyze ER-118585 and its metabolites, sets of samples were placed in an autosampler and stored them at 4 °C. The samples were injected using on-line dilution in the context of the extraction mobile phase. The solutes were concentrated on the MC-ODS SPE column for 2 min with an extraction mobile phase that contained a mixture of 20 mmol/L of ammonium acetate (pH 7) and acetonitrile (90:10, v/v). Following the removal of the plasma proteins and other matrix components, the target compounds enriched on the MC-ODS SPE column were transferred to the analytical column by switching a six-port valve into gradient elution mode at a flow rate of 1.0 mL/min. The analytes were detected by SPD-10Avp UV detector at a wavelength of 277 nm. The mobile phases consisted of (A) a mixture of 20 mmol/L of ammonium acetate (pH 7) and acetonitrile (90:10, v/v) and (B) a mixture of 20 mmol/L of ammonium acetate (pH 7) and acetonitrile (10:90, v/v).

The peak areas obtained were plotted against the concentrations of the analytes (expressed as μg/mL) and the calibration curves constructed by means of the least-square method. Intra- and inter-day accuracy and precision were evaluated ($n = 3$ for intra-day and $n = 6$ for inter-day) in the validation experiments.

2.8. Simultaneous determination of ER-118585 and its metabolites in plasma after continuous infusion of ER-118585 into a canine specimen

Male beagle dogs (Marshall Farms, NY, US) weighing 10 kg were fed restricted diets. ER-118585 (1 and 10 μg/(kg min)) in a 5% glucose solution containing 5 mmol/L phosphoric acid was infused continuously (0.3 mL/min) into the cephalic vein over a period of 60 min for each dose. Blood samples of about 1.5 mL were drawn from the femoral artery using a heparinized syringe at 30, 60, 90 and 120 min following ER-118585 administration. The blood samples were centrifuged to obtain the plasma, which was subsequently stored at −20 °C.

3. Results and discussion

3.1. On-line extraction of model drugs from human plasma

In order to evaluate drug recovery from plasma after direct injection into the MC-ODS SPE column using normal column-switching HPLC, drug recoveries from 100 μL of plasma sample spiked with ketoprofen (pK_a; 4.29 [26], log $P_{(o/w)}$; 3.12 [27]), propranolol (pK_a of conjugated acid; 9.59 [28], log $P_{(o/w)}$; 3.56 [29]) or imipramine (pK_a; 9.58 [28], log $P_{(o/w)}$; 4.80 [29]) were evaluated. As a plasma matrix, human plasma, in which drug–protein binding was well studied, was used.

Better than 90% recovery rates were obtained for propranolol and imipramine, but the recovery rate for ketoprofen was 28%. The effectiveness of recovery of these drugs from standard solutions reached almost 100%. Diluting the plasma sample with phosphate buffer saline improved the recovery of 100 ng of ketoprofen; the recovery from 20 μL of a 2× diluted sample and 100 μL of a 10× diluted sample was 50 and 95%, respectively. It was assumed that the low drug recovery rates might be due to the presence of plasma matrices.

In human plasma, human serum albumin (HSA) comprises more than 50% of the plasma protein and plays an important role in drug–protein binding in addition to α_1-acid glycoprotein (α_1-AGP). The binding constants of propranolol and imipramine to HSA are 6.6×10^4 and 2.4×10^2 M^{-1}. The binding constants of these drugs with α_1-AGP are 4.8×10^5 and 9.2×10^4 M^{-1} [30,31], indicating stronger binding to α_1-AGP in comparison with HSA. However, these values were not very large and in the on-line extraction of these drugs after plasma direct injection these drugs could be extracted with satisfactory rates of recovery. On the other hand, the binding constant of ketoprofen to HSA is 1.6×10^6 M^{-1} [32]. In on-line extraction from plasma using the normal column-switching system, it was thought that the addition of an organic solvent and a reduction in pH of the extraction mobile phase might weaken the drug–protein binding. Unfortunately, however ketoprofen might possibly exist in nonionic form and showed strong binding to albumin, consequently a sufficient rate of recovery was not obtained.

Hence, the effect of the extraction flow rate and injection volume on the ability to recover ketoprofen and propranolol from plasma was investigated. As shown in Fig. 2, the drug recovery rates improved with lower flow rates of the extraction mobile phase. A notable effect was observed for ketoprofen. It was found that smaller plasma injection volumes led to higher recovery rates for both drugs (Fig. 2). It was confirmed that the reduction of protein concentrations in the extraction mobile phase is effective to obtain improved drug recovery in the on-line extraction protocol.

It was concluded from the aforementioned data that, in order to achieve a sufficient recovery of the drug from plasma, it is important to reduce the plasma concentration and lower the mobility of the drugs in the extraction column. However, lowering the flow rate of the extraction mobile phase lengthened the extraction time and resulted in a longer total analysis time. Accordingly, in order to achieve both a reduction in plasma concentration and the lowering of the mobility of the drugs in the column, a column-switching HPLC apparatus that features a bypass line in front of and behind the injector [24] and another column-switching HPLC apparatus with a dilution line in the extraction system (Fig. 1) were developed.

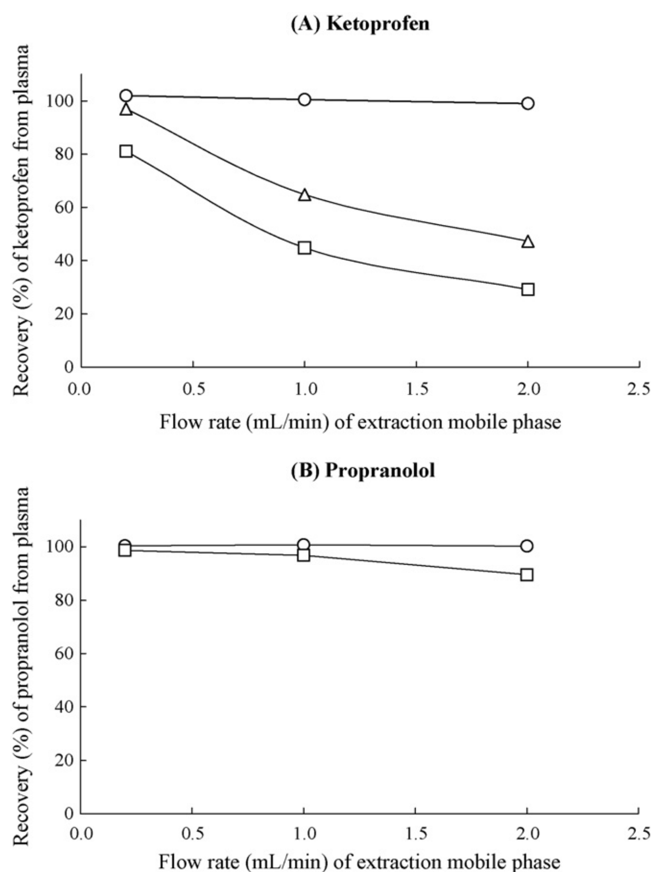

Fig. 2. Effect of varying the extraction flow rate and selecting injection volumes of (○) 10 μL; (△) 50 μL; (□) 100 μL on drug recovery from plasma using the MC-ODS SPE column. Conditions were as follows: extraction mobile phase, 20 mmol/L phosphoric acid (pH 2) – acetonitrile (90:10) for ketoprofen and 20 mmol/L phosphate buffer (pH 7) – acetonitrile (90:10, v/v) for propranolol; mobile phase volume: 4 mL; drug amount: 100 ng.

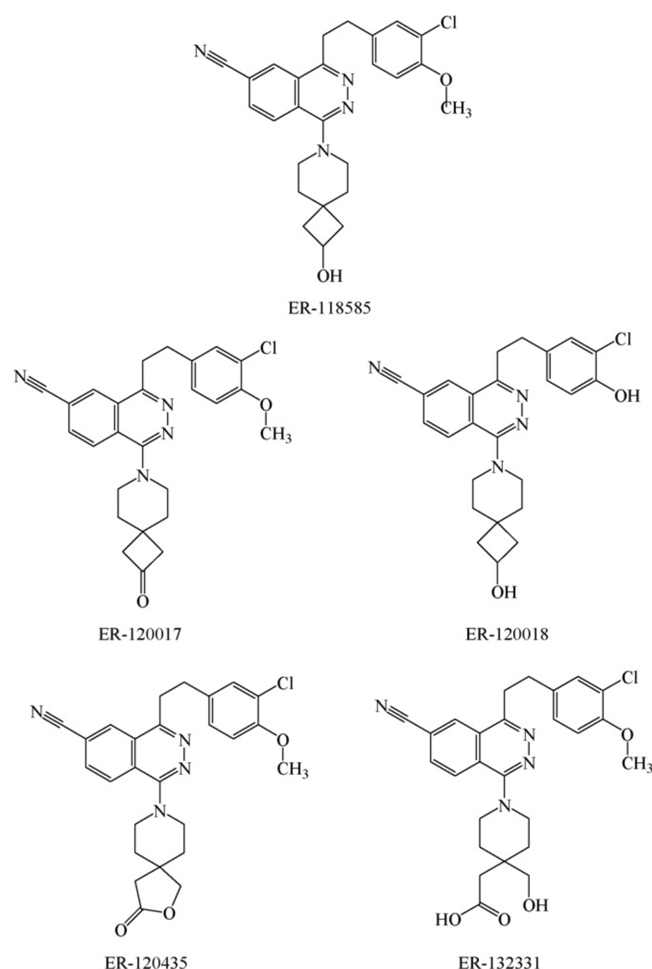

Fig. 4. Structure of ER-118585 and its metabolites (ER-120017, ER-120018, ER-120435 and ER-132331).

The effect of the extent of on-line dilution of the plasma sample on the recovery rates of drugs from 100 μL of plasma was investigated. With dilution ratios from 6 to 8, the obtained recovery rates were nearly 100% for the model drugs tested (Fig. 3). If the dilution ratio were too large, it would take more time to transfer the sample to the MC-ODS column, which in turn would lead to the elution of the analytes from the MC-ODS column. Finally, it would result in a longer extraction time and reduced HPLC sensitivity. A report describing one such on-line dilution was helpful to improve the sensitivity of the method [33].

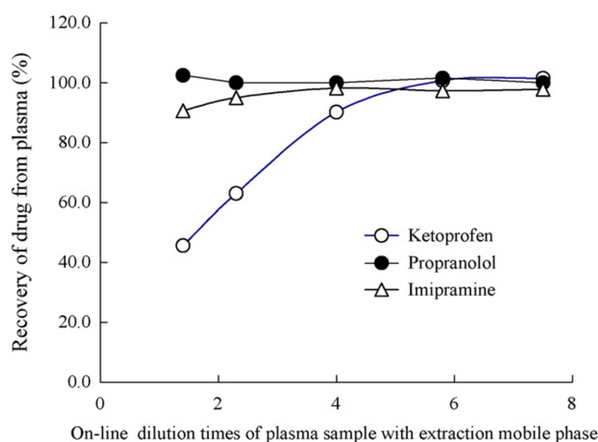

Fig. 3. Effect of on-line dilution on drug recovery from plasma using the MC-ODS SPE column. Extraction conditions were as follows: mobile phase: 20 mmol/L phosphate buffer (pH 2) – acetonitrile (90:10) for ketoprofen and 20 mmol/L phosphate buffer (pH 7) – acetonitrile (90:10, v/v) for propranolol and imipramine; extraction time, 4 min; flow rate: 1 mL/min; sample: 1 μg/mL, 100 μL.

3.2. Simultaneous determination of ER-118585 and its metabolites in beagle dog plasma

In order to confirm the real-world applicability of the column-switching HPLC protocol, the simultaneous determination of ER-118585 and its metabolites in beagle dog plasma was investigated. ER-118585 is a hydrophobic basic compound with $\log P_{(o/w)}$ 4.3. When ER-118585 is administrated to a mammal, it produces four metabolites, namely ER-120017 (ketone metabolite), ER-120018 (desmethyl metabolite), ER-120435 (lactone metabolite) and ER-132331 (carboxylate metabolite). The chemical structures of these compounds are shown in Fig. 4.

These compounds are hydrophobic and are associated with large albumin binding constants, especially the carboxylate. To lower the hydrophobic interactions between the compound-protein and to achieve acceptable rates of on-line extraction of these compounds, a mixture of 20 mmol/L ammonium acetate (pH 7) and acetonitrile (90:10, v/v) was employed as the extraction mobile phase.

The effect of the on-line dilution ratio on the recovery rates for ER-118585 and its metabolites was investigated. An 8× on-line dilution protocol resulted in almost 100% recovery of all of the com-

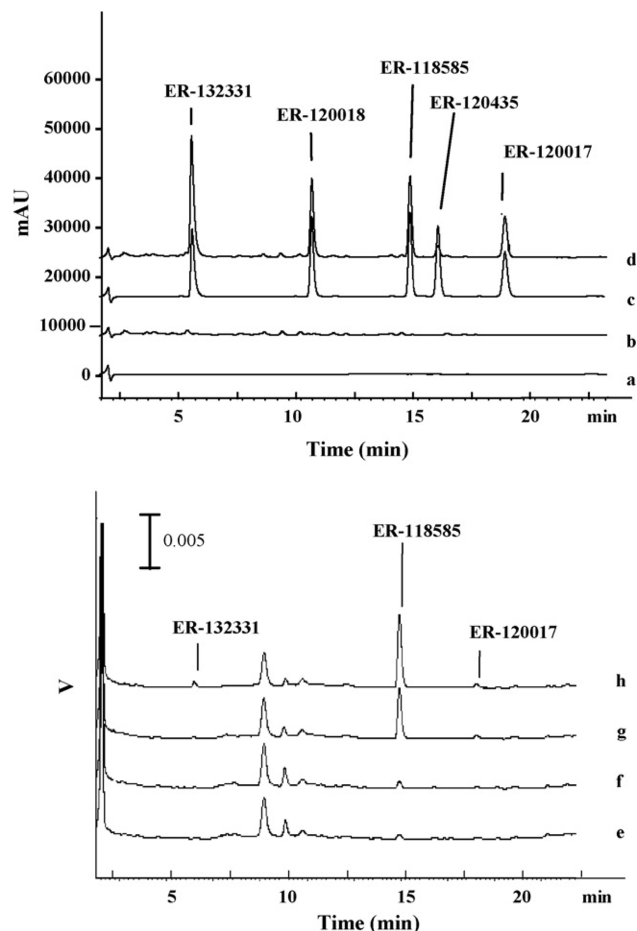

Fig. 5. Representative HPLC chromatograms of ER-118585 and its metabolites: (a) blank (30% acetonitrile), (b) blank plasma, (c) standard mixture (ER-118585, ER-120017, ER-120018, ER-120435 and ER-132331), (d) plasma supplemented with test compounds, beagle plasma after (e) 30 min, (f) 60 min, (g) 90 min and (h) 120 min IV infusion of ER-118585. The concentration of each compound in (c) and (d): 250 ng/mL. Infusion condition in (e–h): 1 μg/(kg min) (0–60 min) and 10 μg/(kg min) (60–120 min).

pounds from the plasma sample. As shown in the representative HPLC chromatograms of a standard solution and a plasma sample supplemented with the aforementioned compounds (250 ng/mL) (Fig. 5c and d), the simultaneous determination of ER-118585 and its metabolites was feasible. Furthermore, separation of each analyte from the background peaks was achieved, and it was confirmed that the background does not significantly influence the quantitative power of this method.

On the other hand, the peak area of ER-120435 in the plasma sample, which was analyzed immediately after preparation, was about 17% of the standard solution. In contrast, the peak area of ER-132331, which exists in equilibrium with ER-120435, was about 184% of the standard reference. Lactone is converted to its carboxylate in a pH-dependent equilibrium [34]. This conversion occurs under neutral and basic conditions and appears to be largely dependent on the presence of hydroxide ions. In plasma, the conversion of ER-120435 to ER-132331 is known to occur. Therefore, the total amount of ER-120435 and ER-132331 can be determined simply by quantifying the ER-132331.

Stability test results for ER-118585 and its metabolites in beagle dog plasma at 4 °C showed that ER-120017 decomposed and the residual percentages after 1 and 5 h of storage were 85 and 55%, respectively. Based on these results, the method to determine ER-118585, ER-120017, ER-120018 and ER-132331 was validated using inactivated plasma that had been treated at 60 °C for 30 min. In

Table 1
Intra- and inter-day precision and accuracy for ER-118585 and its metabolites in plasma sample.

Spiked concentration (ng/mL)	ER-132331			ER-120018			ER-118585			ER-120017		
	Found concentration (ng/mL)	CV (%)	Accuracy (%)	Found concentration (ng/mL)	CV (%)	Accuracy (%)	Found concentration (ng/mL)	CV (%)	Accuracy (%)	Found concentration (ng/mL)	CV (%)	Accuracy (%)
Intra-day												
25	22.6 ± 1.9	8.4	−9.7	27.8 ± 1.3	4.8	11.1	28.7 ± 0.9	3.0	14.8	28.8 ± 0.9	3.0	15.4
50	52.5 ± 4.1	7.7	5.1	49.4 ± 3.7	7.5	−1.2	54.5 ± 2.4	4.4	8.9	55.5 ± 2.7	4.8	11.0
250	259.2 ± 2.7	1.0	3.7	273.0 ± 23.6	8.7	9.2	260.0 ± 3.5	1.4	4.0	222.5 ± 12.9	5.8	−11.0
500	462.3 ± 11.1	2.4	−7.5	513.5 ± 8.3	1.6	2.7	504.9 ± 1.1	0.2	1.0	497.5 ± 5.1	1.0	−0.5
2500	2431.0 ± 99.8	4.1	99.8	2514.5 ± 8.7	0.3	0.6	2511.5 ± 10.9	0.4	0.5	2426.2 ± 24.4	1.0	−3.0
Inter-day												
25	22.5 ± 1.4	6.1	−10.0	28.3 ± 1.2	4.3	13.1	28.4 ± 0.8	2.7	13.4	28.0 ± 1.4	5.0	12.0
50	51.4 ± 3.4	6.5	2.9	50.3 ± 2.9	5.8	0.6	54.6 ± 2.3	4.2	9.1	54.2 ± 2.6	4.8	8.4
250	245.0 ± 19.6	8.0	−2.0	265.6 ± 19.6	7.4	−2.0	254.2 ± 8.4	3.3	1.7	235.7 ± 21.4	9.1	−5.7
500	483.2 ± 29.7	6.1	−3.4	506.5 ± 11.3	2.2	1.3	504.1 ± 1.5	0.3	0.8	503.0 ± 8.4	1.7	0.6
2500	2417.4 ± 106.1	4.4	−3.3	2516.7 ± 6.9	0.3	0.7	2517.5 ± 12.4	0.5	0.7	2502.7 ± 106.2	4.2	0.1

$n = 3$ for intra-day and $n = 6$ for inter-day.

practice, however, it is impossible to apply such a sample pretreatment to the routine analysis.

Calibration curves were obtained by plotting the peak area against the compound concentration. These curves were linear over concentrations of 0.025–2.5 μg/mL, with coefficient of determination (r^2) that exceeded 0.999 for every compound. The equations for the calibration plots were $y = 42295.2x - 368.5$ for the ER-132331 standard, $y = 39026.0x - 29.7$ for ER-132331 in plasma, $y = 61979.9x - 615.0$ for the ER-120018 standard, $y = 62258.5x - 29.7$ for ER-120018 in plasma, $y = 54013.5x - 316.1$ for the ER-118585 standard, $y = 53770.6x + 152.1$ for ER-118585 in plasma, $y = 51999.8x - 514.7$ for the ER-120017 standard, and $y = 51117.0x - 762.4$ for ER-120017 in plasma.

The intra- and inter-day precision and accuracy were evaluated by analyzing beagle dog plasma that had been supplemented with 25, 50, 250, 500 and 2500 ng/mL of the four compounds. The intra- and inter-day accuracy and precisions for ER-118585 and its metabolites were satisfactory as shown in Table 1. The background peaks in the HPLC chromatograms slightly affected the accuracy of the method at 25 ng/mL for all compounds. However, the validation data met the established criteria [35]. It was concluded that the on-line extraction of ER-118585 and its metabolites using the MC-ODS SPE column methodology could serve as a reproducible assay for analysis of beagle dog plasma with lower limit of quantification of 25 ng/mL. These data confirm the ability to prepare on-line samples that contain both acidic and basic compounds in plasma using the MC-ODS SPE column in the context of the column-switching HPLC approach.

3.3. Application to the simultaneous determination of ER-11858 and its metabolites in plasma after continuous infusion of ER-118585 to a canine specimen

To confirm the applicability of the procedures, the concentrations of ER-118585 and its metabolites in plasma samples obtained after continuous infusion of ER-118585 to canines of the beagle strain were determined. Representative HPLC chromatograms of beagle plasma following continuous IV infusion of an ER-118585 solution for 30, 60, 90 and 120 min were presented in Fig. 5e–h. ER-132331 and ER-120017 were detected in the HPLC chromatograms of the plasma sample obtained after infusion of ER-118585 at 10 μg/(kg min). In the chromatograms obtained in the study, endogenous components might affect the specificity for ER-120018 peak were observed. The resulting concentrations for ER-118585 in beagle plasma after 30, 60, 90 and 120 min infusion were 59.3, 87.8, 658.6 and 955.4 ng/mL. The concentrations of ER-120017 were determined as 46.2 and 58.6 ng/mL after 90 and 120 min infusion and that of ER-132331 was 76.5 ng/mL after 120 min infusion, respectively. It was concluded that the method has an ability to determine ER-118585 and its metabolites in beagle plasma.

4. Conclusion

Effective on-line extraction of acidic and basic drugs in plasma using a MC-ODS RAM SPE column in column-switching high performance liquid chromatography (HPLC) equipped with an on-line dilution system was investigated. It was found that on-line dilution of the plasma sample in the mobile phase improves the extraction efficacy of drugs in plasma. Furthermore, with a six- to eightfold dilution ratio, almost 100% recovery rates were obtained for model acidic and basic drugs from plasma samples. The proposed extraction procedure was validated by applying it to the simultaneous determination of ER-118585 and its metabolites. The real-world applicability of the method was successfully demonstrated by the determination of ER-118585 and its metabolites in a batch of test samples.

References

[1] C.P. Desilets, M.A. Rounds, F.E. Regnier, J. Chromatogr. 544 (1991) 25–39.
[2] N.M. Cassiano, V.V. Lima, R.V. Oliverira, A.C.D. Piatro, Q.B. Cass, Anal. Bioanal. Chem. 384 (2006) 1462–1469.
[3] S.S. Singh, M. Jain, H. Shah, S. Gupta, P. Thakke, R. Shah, B.B. Lohray, J. Chromatogr. B 813 (2004) 247–254.
[4] Y. Asakawa, C. Ozawa, K. Osada, S. Kaneko, N. Asakawa, J. Pharm. Biomed. Anal. 43 (2007) 683–690.
[5] T. Sakaguchi, E. Yamamoto, I. Kushida, T. Kajima, N. Asakawa, J. Pharm. Biomed. Anal. 40 (2006) 345–352.
[6] S. Kawano, M. Takahashi, T. Hine, E. Yamamoto, N. Asakawa, Rapid Commun. Mass Spectrom. 19 (2005) 2827–2832.
[7] H. Yoshida, I. Morita, G. Tamai, T. Masujima, T. Tsuru, N. Takai, H. Imai, Chromatographia 19 (1985) 466–472.
[8] D. Ohman, B. Carlsson, B. Norlander, J. Chromatogr. B, Biomed. Sci. Appl. 753 (2001) 365–373.
[9] M. Schlauch, K. Fulde, A.W. Frahm, J. Chromatogr. B 775 (2002) 197–207.
[10] V.K. Boppana, W.H. Schaefer, M.J. Cyronak, J. Biochem. Biophys. Methods 54 (2002) 315–326.
[11] R. Brunetto, L. Gutiérrez, Y. Delgado, M. Gallignani, J.L. Burguera, M. Burguera, Anal. Bioanal. Chem. 375 (2003) 534–538.
[12] A.J. Santos-Neto, J. Bergquist, F.M. Lancas, P.J.R. Sjoberg, J. Chromatogr. A 1189 (2008) 514–522.
[13] P. Chiap, A. Ceccato, R. Gora, P. Hubert, J. Geczy, J. Crommen, J. Pharm. Biomed. Anal. 27 (2002) 447–455.
[14] J. Haginaka, H. Sanbe, Anal. Chem. 72 (2000) 5206–5210.
[15] S.O. Choi, S.Y. Um, S.H. Jung, S.J. Jung, J.I. Kim, H.J. Lee, S.Y. Chung, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 830 (2006) 301–305.
[16] J. Zhang, D.G. Musson, K.L. Birk, A.M. Cairns, A.L. Fisher, W. Neway, J.D. Rogers, J. Pharm. Biomed. Anal. 27 (2002) 755–770.
[17] W.R.G. Baeyens, G. van der Weken, J. Haustraete, H.Y. Aboul-Enein, S. Corveleyn, J.P. Remon, A.M. García-Campaña, P. Deprez, Biomed. Chromatogr. 13 (1999) 450–454.
[18] P. Chiap, O. Rbeida, B. Christiaens, P. Hubert, D. Lubda, K.S. Boos, J. Crommen, J. Chromatogr. A 975 (2002) 145–155.
[19] O. Rbeida, B. Christiaens, P. Hubert, D. Lubda, K.S. Boos, J. Crommen, P. Chiap, J. Chromatogr. A 1030 (2004) 95–102.
[20] E. Yamamoto, T. Sakaguchi, T. Kajima, N. Mano, N. Asakawa, J. Chromatogr. B 807 (2004) 327–334.
[21] E. Yamamoto, S. Takakuwa, T. Kato, N. Asakawa, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 846 (2007) 132–138.
[22] Y. Sato, E. Yamamoto, S. Takakuwa, T. Kato, N. Asakawa, J. Chromatogr. A 1190 (2008) 8–13.
[23] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 137–147.
[24] E. Yamamoto, K. Murata, Y. Ishihama, N. Asakawa, Anal. Sci. 17 (2001) 1155–1159.
[25] H. Mizuno, H. Adachi, J. Kimura, Y. Sawa, M. Kakiki, K. Lansdell, M. Saito, W.D. Kerns, Biol. Pharm. Bull. 26 (2003) 1661–1667.
[26] M. Meloun, S. Bordovská, Anal. Bioanal. Chem. 389 (2007) 1267–1281.
[27] P. Wiczling, P. Kawczak, A. Nasal, R. Kaliszan, Anal. Chem. 78 (2006) 239–249.
[28] M. Shalaeva, J. Kenseth, F. Lombardo, A. Bastin, J. Pharm. Sci. 97 (2008) 2581–2606.
[29] I. Moriguchi, S. Hirono, I. Nakagomi, H. Hirano, Chem. Pharm. Bull. 42 (1994) 976–978.
[30] J.M. Kremer, J. Wilting, L.H. Jansen, Pharmacol. Rev. 40 (1988) 1–47.
[31] U. Kragh-Hansen, Pharmacol. Rev. 33 (1981) 17–53.
[32] H. Wang, Z. Wang, M. Lu, H. Zou, Anal. Chem. 80 (2008) 2993–2999.
[33] M. Liu, W. Yan, J.-M. Lin, Y. Hashi, L.-B. Liu, Y. Wei, J. Chromatogr. A 1198–1199 (2008) 87–94.
[34] J. Fassberg, V.J. Stella, J. Pharm. Sci. 81 (1992) 676–684.
[35] Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research (CBER), 2001.