ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 405–410

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Direct determination of verapamil in rat plasma by coupled column microbore-HPLC method

O.H. Jhee[a], J.W. Hong[b], A.S. Om[a], M.H. Lee[c], W.S. Lee[b,d], L.M. Shaw[e], J.-W. Lee[b], J.S. Kang[b,*]

[a] *Department of Food and Nutrition, College of Human Ecology and Institute of Biomedical Science, Hanyang University, Seoul 133-791, South Korea*
[b] *Department of Pharmacology, College of Medicine and Institute of Biomedical Science, Hanyang University, Seoul 133-791, South Korea*
[c] *Department of Internal Medicine, College of Medicine, Hanyang University, Seoul 133-791, South Korea*
[d] *Scientific Affairs Department, Sanofi-Synthelabo Korea Co. Ltd., Seoul 135-932, South Korea*
[e] *Department of Pathology and Lab medicine, University of Pennsylvania Medical Center, Philadelphia, PA 19104, USA*

Received 6 August 2004; received in revised form 29 October 2004; accepted 1 November 2004
Available online 8 December 2004

Abstract

This report describes an automated coupled column microbore-high-performance liquid chromatography (HPLC) with fluorescence detection for direct determination of verapamil in small volume of rat plasma. We used HPLC system consisting of three columns such as precolumn, intermediate and analytical column and six-port switching valve and injected small volume of rat plasma to the system without sample preparation. An aliquot of sample was directly injected into Capcell Pak MF Ph precolumn for clean-up and enrichment, 35 mm Capcell Pak C18, intermediate column for concentration of compounds and 250 mm Capcell Pak C18 analytical column for separation of compounds and two mobile phases are used as mobile phase A (50 mM ammonium phosphate, pH 4.5) and B (50 mM ammonium phosphate:acetonitrile = 70:30 v/v). Analysis of verapamil and internal standard, propranolol was performed with direct injection of 10 μl of rat plasma to the system and were eluted at 22 and 12 min, respectively, at a mobile phase flow rate of 0.5 (mobile phase A) and 0.15 ml/min (mobile phase B). The peaks of verapamil and internal standard were good shapes and well separated from any interfering endogenous peaks during a total run time of 25 min. The calibration curve for verapamil showed good linearity ($r^2 = 0.9997$) over the concentration range of 0.01–2.50 μg/ml. The mean RSD (%) values of intra-day ($n = 5$) and inter-day ($n = 5$) variability of verapamil ranged from 1.96 to 9.06 and 0.62 to 3.08%, respectively. The LOD and LOQ were 0.01 and 0.025 μg/ml, respectively, for verapamil using 10 μl of rat plasma. An automated coupled column microbore-HPLC method was successfully applied to a pharmacokinetic study after intravenous injection of 3 mg/kg of verapamil to the normal and dimethylnitrosamine (DMN)-induced hepatofibrotic rats.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Verapamil; Propranolol; Coupled column; Automated microbore-HPLC; Fluorescence detector; Rat plasma; DMN-induced hepatofibrotic rat

1. Introduction

Verapamil (Fig. 1), a synthetic paraverin derivate, which belongs to phenylalkylamine class, is a calcium blocker [1]. It has been used an important therapeutic agent for angina pectoris, ischemic heart disease, hypertension and hypertrophic cardiomyopathy [2]. For clinical purpose two types of oral dosage of verapamil (40–180 and 120–140 mg in the case of conventional tablets and slow releasing tablets, respectively) have been mostly used. Despite of 90% of oral absorption rate, low oral bioavailability (only 10–20%) of verapamil is attributed by extensive hepatic first-pass effect leading to a pharmacologically inactivation. During the metabolism it converted into norverapamil (*N*-demethylated metabolite), which exerts 20% pharmacological activity of verapamil, and other inactive metabolites [3]. In pharmacokinetic study of verapamil therapeutic plasma levels were

* Corresponding author. Tel.: +82 2 2290 0652; fax: +82 2 2292 6686.
E-mail address: jskang@hanyang.ac.kr (J.S. Kang).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.11.003

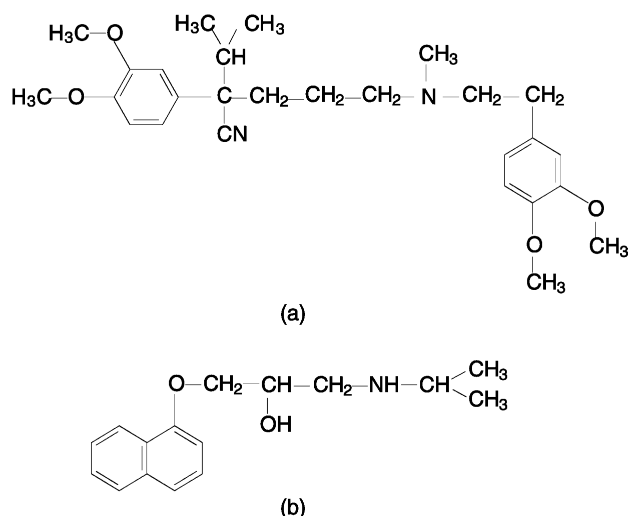

Fig. 1. Structures of verapamil (a) and propranolol (b); internal standard.

considerably variable from 20 to 500 ng/ml depending on drug form used [4]. This suggests that patients require frequent adjustments of individual dosage of this drug. For analysis of drugs and their metabolites in biological samples, an efficient high-performance liquid chromatography (HPLC) combined with a various mode of detection, which is complicate to use, has been used. Because of undesirable sensitivity and selectivity, sample preparations including sample clean-up, and pre-concentrations are required prior to analysis [5]. Traditional sample preparation methods such as liquid–liquid extraction and solid-phase extraction (SPE) are time-consuming and imprecise. The case of liquid–liquid extraction is difficult to be automated unlike the SPE method that is extremely flexible except for requirement of complex of robotic devices. Also, both liquid–liquid extraction and SPE methods require large amounts of high-purity solvents [6]. In contrast using an automated one-line coupled column or column-switching devices coupled advanced separation media technologies are enable to perform automated clean-up and trace enrichment of analytes in biological samples as well as enable to improve analytical process [5]. Various analytical techniques have been introduced for quantifying verapamil in biological fluids such as plasma, serum and urine, as well as tissues [7]. To date, HPLC method has been mainly employed to quantify the concentrations of verapamil in biological samples [8], even though gas chromatography coupled nitrogen–phosphorous detector has been widely used for measuring trace levels of drugs [9]. It has been reported that verapamil in biological fluids and tissue homogenates was determined by a reproducible fluorometric method [7]. However, this method requires a very large volume of sample (greater than 4 ml), complicated sample preparation and interfering detection by fluorescent inactive several metabolites. More sensitive and specific approaches to measure plasma verapamil are mass spectrometry (MS) with isotope dilution (mass fragmentography) [10] and HPLC/MS [11]. But, these techniques have limitations including requirement of expensive instruments and spacious laboratory. In addition, gas liquid chromatography has been successfully applied to analysis of verapamil and some of its metabolites in biological fluids [12]. But it is not appropriate to detect verapamil in micro-volume of plasma, because this method also requires at least 1 ml of plasma samples. Furthermore, HPLC using either reversed-phase [13], or normal phase [14], or ion-exchange column [15] with spectrometric detection [16] have been described. In these methods isolation of varapamil from biological fluids was performed by liquid–liquid extraction that is extraction from alkaline sample into a volatile, water-immiscible, and organic phase followed by evaporation, subsequently reconstitution into mobile phase, or solvent compatible with mobile phase [17]. However, the initial extraction is followed by a back-extraction into dilute aqueous acid that is then injected onto the column [15].

The purpose of this study is to develop and validate a fully automated reverse-phase coupled column microbore-HPLC method with fluorometric detector for measuring verapamil in micro-volume (10 μl) without sample preparation. The procedures includes precolumn for clean-up, enrichment of compounds followed by intermediate column for sample concentration or extraction, finally transferred to the analytic column for subsequent HPLC analysis by means of an automated column-switching valve. To confirm the feasibility of the method we tried to apply this method to assess the pharmacokinetic properties of verapamil in hepatofibrotic rat models.

2. Experimental

2.1. Chemicals and solutions

Dimethylnitrosamine, verapamil hydrochloride and propranolol hydrochloride (I.S., internal standard) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and purity of these materials was greater than 99.5%. Ammonium phosphate dibasic (Wako Pure Chemical Industries Ltd., Japan) and pentobarbital sodium salt (TCI, Tokyo, Japan) were analytical reagent grade. Acetonitrile (HPLC grade) was purchased from Merck Co. (Darmstadt, Germany). Water was deionised and purified using a Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of verapamil and propranolol containing 1.0 and 10.0 mg/ml of each products in mobile phase A were prepared and stored at −20 °C. Stock standard solutions of verapamil were prepared at a concentration of 1 mg/ml in mobile phase and diluted at a concentration of 1 μg/ml using the same solvent; the resulting solution, was stable for several weeks at 4 °C. Under these conditions, the solution was stable for several weeks. Working standard solutions of verapamil were prepared by sequential dilution by drug-free plasma at 10, 25, 50, 250, 500, 1000 and 2500 ng/ml.

2.2. HPLC apparatus and chromatographic conditions

The coupled column HPLC system used was a NANOSPACE SI-1 microbore system equipped with a six-port switching valve unit (Shiseido Co., Tokyo, Japan). The system is designed for semi-microcolumn LC by reducing any possible dead spaced volume in the entire system because the dead volumes in the connections between columns and any switching valves can negatively affect the separation efficiency. The column used for the sample clean-up step was an MF-Ph precolumn, Capcell Pak (4.0 mm i.d. × 20 mm, Shiseido Co., Tokyo, Japan). A C-18 column, Capcell Pak UG120 (2.0 mm i.d. × 35 mm, Shiseido Co., Tokyo, Japan) was used for the primary separation of compound from plasma using the mobile phase A at 0.5 ml/min; an analytical C-18 column, Capcell Pak UG120 (1.5 mm i.d. × 250 mm, Shiseido Co., Tokyo, Japan) was employed in the analysis of verapamil by using the mobile phase B at a flow rate of a 0.15 ml/min. The column temperature was maintained constant at 45 °C. The column effluent was monitored using a fluorescence detector with an excitation wavelength of 280 nm and an emission wavelength of 313 nm. Mobile phase A (50 mM ammonium phosphate, pH 4.5) and mobile phase B (50 mM ammonium phosphate:acetonitrile = 70:30 v/v) were filtered and degassed through a 0.22 μm Magna-R filter (Whatman International Co., Maidstone, UK).

2.3. Analytical procedures

A diagram of the different column-switching positions and time sequences of column-switching procedure in the HPLC system is shown in Table 1, respectively.

- Step 1 (0–2.35 and 3.10–3.83 min, Table 1 and Fig. 2). Plasma sample (10 μl) was introduced onto precolumn where plasma proteins, verapamil and internal standard were separated using mobile phase A at flow rate of 0.5 ml/min. The intermediate column and analytical column were equilibrated using mobile phase B at a flow rate of 0.15 ml/min.
- Step 2 (2.35–3.10 and 3.85–4.85 min, Table 1 and Fig. 2). When the valve status was changed to B, target drug-containing fraction separated in precolumn was focused on to the top an intermediate column using mobile phase A at flow rate of 0.5 ml/min. The analytical column was equilibrated using mobile phase B.
- Step 3 (4.85–25.0 min, Table 1 and Fig. 2). The compounds trapped in the top of intermediate column were transferred to the analytical column and analyzed by fluorescence detector at an excitation wavelength of 280 nm and an emission wavelength of 313 nm.

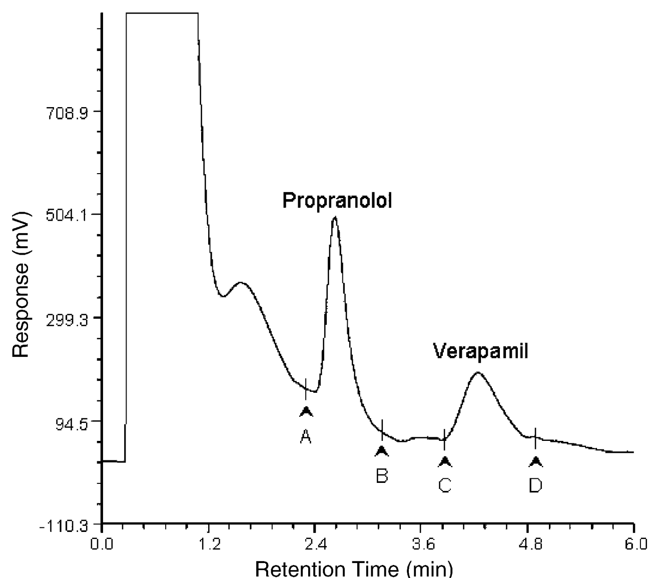

Fig. 2. Separation of verapamil and propranolol-spiked plasma on MF Ph-1 precolumn and determination of switching time (min) for valves (⮝: 2.35, 3.10, 3.85 and 4.85); conditions: mobile phase A, flow rate: 0.5 ml/min, injection volume: 10 μl.

2.4. Method validation

The calibration curve and linearity for verapamil were investigated by diluting aliquots of the working standards with normal rat plasma to obtain concentrations of 0.01, 0.025, 0.10, 0.25, 0.5, and 1.0 μg/ml of verapamil and 400 ng/ml of propranolol to drug-free rat plasma and the mixtures diluted as same volume by mobile phase A. After dilution the samples was centrifuged at 13 000 × g for 10 min to spin down the precipitate, the supernatant loaded into autosampler and 10 μl were injected into the HPLC system. For the analysis, aliquots of 0.4 ml of plasma were supplemented with 40 μl of a propranolol (10.0 μg/ml) and subsequently pro-

Table 1
Time sequences of column-switching procedure in analytical process

Switching time (min)	Valve status	Mobile phase	Flow rate (μl/min)	Event
0–2.35, 3.10–3.85	A	A	500	Plasma sample introduced into precolumn where proteins and compounds were separated and sample fraction without analytes into the waste bypass intermediate column, analytical column and detector
2.35–3.10, 3.85–4.85	B	A	500	The fraction of analytes were introduced and trapped in the top of intermediate column
4.85–25.0	C	B	150	The compounds trapped in the intermediate column were transferred and analyzed in the analytical column

Mobile phase A: 50 mM ammonium phosphate, pH 4.5; mobile phase B: 50 mM ammonium phosphate (pH 4.5):acetonitrile = 70:30 v/v.

cessed following the method described above. The calibration curves were obtained by plotting the ratio of the peak areas of verapamil to internal standard against the concentrations of verapamil spiked into drug-free rat plasma. Intra-day reproducibility was evaluated by analyzing sets of drug-free rat plasma samples, spiked with four concentrations of verapamil hydrochloride in the range 0.025–2.50 μg/ml at five different time periods. The assessment of inter-day reproducibility was based on the analysis of the same spiked plasma samples on five consecutive days. The limit of detection (LOD) for verapamil was determined as the concentration of drug giving a signal to noise ratio greater than 3. The lower limit of quantitation (LOQ) was determined as the minimum concentration that can be accurately and precisely quantified [18].

2.5. Application to animal studies

Pharmacokinetics studies were carried out using male *Sprague-Dawley* rats (BW, 200–250 g, Joongang Animal Co., Seoul, Korea) divided into groups of five rats each and fasted overnight for 12 h prior to an experiment. The hepatofibrosis was induced by DMN according to George et al. method [19]. Experimental rats received the 1% DMN (1.0 ml/kg i.p.) by injection for three consecutive days per week for a period of 4 weeks and control rats were treated with the same amount of saline. After anesthesia with pentobarbital sodium (30 mg/kg i.p.), the rats were surgically exposed the left femoral artery, of the rats was surgically exposed and cannulated with catheter, intravascular catheterisation (PE-50) for blood sampling purposes. Verapamil (3 mg/kg i.v.) was administered through the tail vein. Blood samples were drawn from the femoral artery at 10, 30, 60, 120 and 180 min after verapamil administration into polyethylene test tubes in each rat, and immediately centrifuged at 1700 × *g* for 15 min. The clear plasma layer was transferred to clean test tubes and stored at −70 °C for verapamil analysis in each plasma samples. For the analysis, aliquots of 0.4 ml of plasma were supplemented with 40 μl of internal standard solution (propranolol, 10.0 μg/ml) and the mixtures diluted as same volume by mobile phase A. After dilution the samples was centrifuged at 13 000 × *g* for 10 min to spin down the precipitate, the supernatant loaded into autosampler and 10 μl were injected into the HPLC system. Plasma levels of verapamil were determined employing the developed automated coupled column microbore-HPLC system with fluorescence detector. Calculation of pharmacokinetic parameters was done using pharmacological calculation systems (Pharm/PCS) version 4.0 (Springer-Verlag, New York, USA, 1986).

3. Results and discussion

Coupled column technique is a useful sample preparations system that can directly analyze complex biological samples in the small volume without any loss in sensitivity and chromatographic efficiency obtained by semi-microcolumns [20,21]. Coupled column devices have been proved to simplify the HPLC analysis of drugs in biological samples, by facilitating the total automation of the chromatographic process, then increasing the speed and work capacity [6]. In this study, Capcell Pak MF Ph-1 precolumn was appropriate to remove proteins and concentrate verapamil and propranolol from plasma. To determine an appropriate time for column-switching, the retention behavior of verapamil and propranolol in plasma on the Capcell Pak MF Ph-1 precolumn was evaluated using mobile phase A and shown in Fig. 2. The constructed coupled column microbore-HPLC with fluorescence detector and time sequences of column procedure is illustrated in Table 1, respectively. HPLC chromatograms obtained from blank plasma, plasma spiked with verapamil (1.0 μg/ml) and propranolol (0.5 μg/ml) and plasma collected from DMN-induced hepatofibrotic rat at 120 min after single intravenous injection of 30 mg/kg of verapamil are shown in Fig. 3. To update, an increasing number of HPLC methods with on-line sample clean-up by solid-phase extraction using coupled column devices have been developed. The principle of coupled column technique for sample clean-up

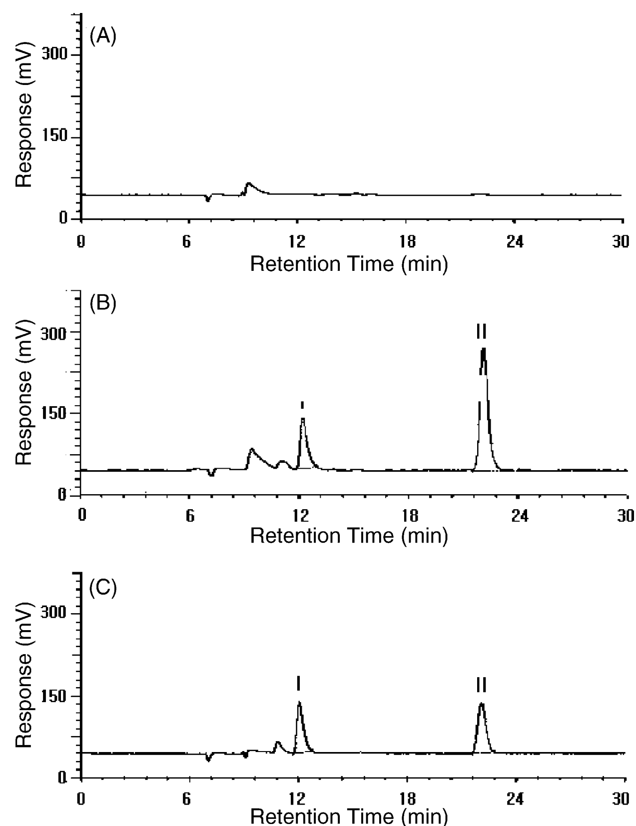

Fig. 3. HPLC chromatograms of: (A) blank plasma, (B) blank plasma spiked with propranolol (0.5 μg/Ml) and verapamil (1.0 μg/Ml), and (C) plasma sample at 2 h after intravenous injection of 30 mg/kg of verapamil and spiked with propranolol (0.5 μg/Ml) to a DMN-induced hepatofibrotic rat (I: propranolol; II: verapamil).

Table 2
Precision and accuracy of coupled column microbore-HPLC method of determination of verapamil in rat plasma

Spiked amount (ng/ml)	Intra-day			Inter-day		
	Measured amount (mean ± S.D., ng/ml)[a]	CV (%)[b]	A.C. (%)[c]	Measured amount (mean ± S.D., ng/ml)	CV (%)	A.C. (%)
25	26.13 ± 2.37	9.06	104.5	24.82 ± 0.51	2.1	99.3
50	56.17 ± 1.1	1.96	112.3	56.14 ± 1.16	2.1	112.3
500	478.57 ± 15.88	3.32	95.7	494.15 ± 8.79	3.1	98.8
2500	2566.79 ± 84.02	3.27	102.7	2528.96 ± 9.01	0.6	101.2

[a] Mean ± S.D. ($n = 5$): concentration were calculated from linear regression equation.
[b] Precision = (S.D./mean) × 100.
[c] A.C. (accuracy, %) = (measured amount/spiked amount) × 100.

is to trap the fraction of the sample that contains the analytes in the precolumn. The other compounds of the biological matrix are eluted to waste, whereas the cut-off effluent containing the analytes is diverted to the intermediate or analytical column, where they are separated for identification and/or quantitation [5]. Zone cutting technique probably is one of the most useful and versatile of the entire coupled column techniques [22]. Therefore, we used two zones cutting techniques for propranolol and verapamil fraction in our system that is shown in Fig. 2. The fractions of verapamil and propranolol isolated from precolumn by fraction cutting techniques were focused in the top of intermediate column to obtain well-separated sharp peaks with retention time of 22 and 12 min in the final separation on analytical column and the obtained chromatograms were shown in Fig. 3. The total analysis process was completed within approximately 25 min. A calibration curve for verapamil quantitation in plasma was obtained by plotting peak area ratios at seven concentrations in the range of 0.01–2.50 μg/ml, in the presence of 0.5 μg/ml of propranolol. The relationship between peak areas ratio and the concentrations showed good linearity ($y(\text{ratio}) = 0.0027 \times (\text{concentration}) + 0.0504$, $r^2 = 0.9997$). To evaluate the precision and accuracy of our method, repeated analysis of verapamil-spiked plasma samples ($n = 5$) was carried out. The intra-day reproducibility was evaluated by comparing the peak area ratios obtained in five different time periods for five sets of plasma serially spiked with verapamil at 0.025, 0.05, 0.5 and 2.5 μg/ml. To assess inter-day reproducibility, the same set of spiked plasma was assayed on three consecutive days. The results are shown in Table 2. The intra-day precision (RSD) was ranged from 1.96 to 9.06%. The inter-day precision (RSD) was ranged from 0.62 to 3.08%. The mean accuracy was 103.3% with RSD of 3.19%. The difference between the measured and the spiked concentration were not more than 13% at any QC con-

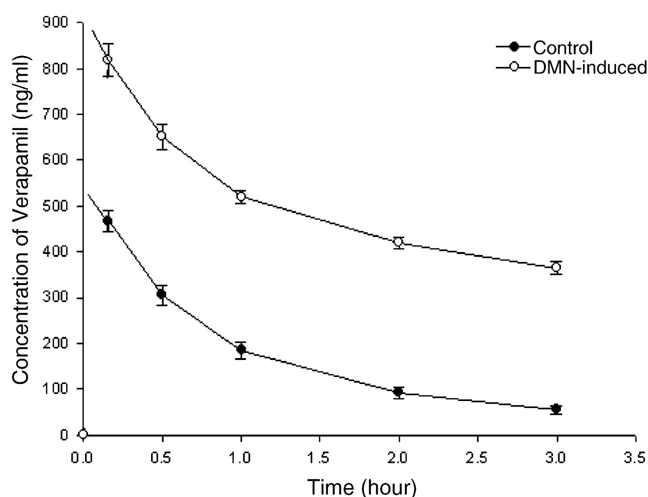

Fig. 4. Plasma mean (±S.D.) concentration–time profiles of verapamil following a single intravenous injection of 30 mg/kg of verapamil in control and DMN-induced hepatofibrotic rats ($n = 10$).

Table 3
Pharmacokinetic parameters (mean ± S.D., $n = 10$) of verapamil in control and DMN-induced hepatofibrotic rats

Parameters	Control	Hepatofibrotic rats	P-value
$AUC_{0\rightarrow 3\,h}$ (ng h/ml)	502.94 ± 39.17	1470.64 ± 39.57	0.01
AUC_{∞} (ng h/ml)	617.73 ± 56.4	1481.55 ± 40.78	0.01
C_{max} (ng/ml)	467.04 ± 22.33	819.10 ± 36.23	0.01
T_{max} (h)	0.16	0.16	NS
Vdss (ml)	2092.89 ± 133.72	897.29 ± 58.02	0.01
CL_p (ml/h)	1469.89 ± 201.45	168.43 ± 18.71	0.01
MRT (h)	1.75 ± 0.31	5.95 ± 0.75	0.01
$t_{1/2(\beta)}$ (h)	1.22 ± 0.22	4.13 ± 0.52	0.01
k_e	0.75 ± 0.15	0.19 ± 0.02	0.01

Statistical significance was calculated by Student's t-test; NS: not significant; AUC: area under the curve; C_{max}: maximum concentration; T_{max}: time to reach to peak serum concentration; MRT: mean residence time; Vdss: steady-state volume of distribution; CL_p: plasma clearance; $t_{1/2(\beta)}$: terminal elimination half-life; k_e: elimination rate constant.

centrations. The LOQ, defined in the presented method as the lowest plasma concentration in the calculation curve that can be measured routinely with acceptance precision (RSD below 20%) and accuracy (80–120%) was 25 ng/ml and LOD, defined in the present method as the concentration of drug giving a signal to noise ratio greater than 3 was 10 ng/ml. Therefore, this coupled column microbore-HPLC method for verapamil was simple and required no sample preparations and a small quantity of a sample was needed to determine verapamil in rat plasma.

The present method was applied to the determination of the pharmacokinetic parameters of verapamil in the plasma of normal and DMN-induced hepatofibrotic rats after intravenous injection of verapamil. Plasma samples were obtained at the 10, 30, 60, 120 and 180 min after verapamil dosing. Fig. 4 was shown the time course of the changes of mean plasma concentration–time profiles of verapamil after dose in control and DMN-induced hepatofibrotic rats. The pharmacokinetic parameters of verapamil in control and DMN-induced hepatofibrotic rats were shown in Table 3. From these results, it is suggested that the present coupled column microbore-HPLC method with fluorescence detector can be applied successfully to analyze verapamil concentration in small volume plasma in the experimental and clinical situations.

4. Conclusions

An automated microbore-HPLC method using coupled column technique without sample preparation has been developed for simple, specific and accurate analysis of verapamil in small volume of rat plasma. The total analysis time of this method was 25 min and sample volume was only 10 μl and superior to other extractive spectrophotometer methods [23,24] in respect to automated simplified, sensitivity, specific and inexpensive experimentation. The suitability of this method was confirmed in the pharmacokinetic study for verapamil in rats.

References

[1] R.N. Brogden, P. Benfield, Drugs 51 (1996) 792–819.
[2] R. Kirsten, K. Nelson, D. Kirsten, B. Heintz, Clin. Pharmacokinet. 34 (1998) 457–482.
[3] M. Walles, W.M. Mullett, K. Levsen, J. Borlak, G. Wunsch, J. Pawliszyn, J. Pharm. Biomed. Anal. 30 (2002) 307–319.
[4] T. Buzinkaiova, I. Skacani, J. Netriova, Pharmazie 50 (1995) 799–805.
[5] P. Campins-Falco, R. Herraez-Hernandez, A. Sevillano-Cabeza, J. Chromatogr. Biomed. Appl. 619 (1993) 177–190.
[6] D.C. Turnell, J.D. Cooper, J. Chromatogr. B: Biomed. Sci. Appl. 492 (1989) 59–83.
[7] R.G. McAllister, S.M. Howell, J. Pharm. Sci. 65 (1976) 431–432.
[8] T. Walle, G.N. Kumar, J.M. McMillan, K.R. Thornburg, U.K. Walle, Biochem. Pharmacol. 46 (1993) 1661–1664.
[9] H.S. Shin, Y.S. Oh-Shin, H.J. Kim, Y.K. Kang, J. Chromatogr. B: Biomed. Sci. Appl. 677 (1996) 369–373.
[10] B. Spiegelhalder, M. Eichelbaum, Arzneimittelforschung 27 (1977) 94–97.
[11] O. von Richter, M. Elchelbaum, F. Schonberger, U. Hofmann, J. Chromatogr. B: Biomed. Sci. Appl. 738 (2000) 137–147.
[12] U.A. Shukla, P.L. Stetson, W.D. Ensminger, J. Chromatogr. 342 (1985) 406–410.
[13] P.H. Hubert, P. Chiap, A. Ceccato, I. Bechet, R. Sibenaler-Dechamps, P. Maes, J. Crommen, J. Pharm. Biomed. Anal. 10 (1992) 937–942.
[14] J.A. Pieper, D.R. Rutledge, J. Chromatogr. Sci. 26 (1988) 474–477.
[15] V.K. Piotrovskii, D.O. Rumiantsev, V.I. Metelitsa, J. Chromatogr. 275 (1983) 195–200.
[16] A.M. Rustum, J. Chromatogr. 528 (1990) 480–486.
[17] B.L. Carter, M.A. Noyes, R.W. Demmler, Pharmacotherapy 13 (1993) 359–368.
[18] S. Afify, U.R. Rapp, P. Hogger, J. Chromatogr. B 809 (2004) 99–103.
[19] J. George, K.R. Rao, R. Stern, G. Chandrakasan, Toxicology 156 (2001) 129–138.
[20] H.S. Lee, J.H. Kim, K. Kim, K.S. Do, Chromatographia 48 (1998) 365–368.
[21] H.M. Lee, S.J. Choi, C.K. Jeong, Y.S. Kim, K.C. Lee, H.S. Lee, J. Chromatogr. B 727 (1999) 213–217.
[22] F. Mangani, G. Luck, C. Fraudeau, E. Verette, J. Chromatogr. A 762 (1997) 235–241.
[23] Y.M. Issa, A.A. Salem, M.S. Bahbouh, Anal. Lett. 30 (1997) 1153–1158.
[24] A.H. Prabhakar, R. Giridhar, V.B. Patel, J. Pharm. Biomed. Anal. 20 (1999) 427–432.