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Short Communication

High-performance liquid chromatographic determination of rifapentine in serum using column switching

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ABSTRACT

A high-performance liquid chromatographic method with column switching has been developed for the determination of rifapentine in serum. The serum samples were injected onto a precolumn packed with Corasil RP C_{18} (37–50 μm) after simple dilution with an internal standard in a 1% ascorbic acid solution. Polar serum components were washed out using 0.05 *M* phosphate buffer. After valve switching, the concentrated drugs were eluted in the back-flush mode and separated by a μBondapak C_{18} column with acetonitrile–tetrahydrofuran–0.05 *M* phosphate buffer (pH 7.0) (42:5:53, v/v/v) as the mobile phase. The method showed excellent precision with good sensitivity and speed, and a detection limit of 0.1 μg/ml. The total analysis time was less than 25 min and the mean coefficients of variation for intra- and inter-assay were less than 4.8%. The method has been successfully applied to serum samples from dogs after the oral administration of rifapentine.

INTRODUCTION

Rifapentine, 3-[(4-cyclopentyl-1-piperazinyl)-iminomethyl]rifamycin SV, is a semisynthetic antibiotic [1]. The antimicrobial spectrum of rifapentine strongly resembles that of its homologue rifapin, with a remarkably greater therapeutic efficacy against *Mycobacterium tuberculosis* and *Mycobacterium leprae* in experimental infection [2].

Pharmacokinetic studies of rifapentine have usually been performed by conventional microbiological assay procedures [3,4]. High-performance liquid chromatography (HPLC) methods have been developed for the determination of rifapentine as a result of advantages such as a short turnaround time, method reliability, sensitivity and specificity [5–7]. All these methods are time consuming and tedious because of the deproteinization or liquid–liquid extraction steps required for sample clean-up. These problems can be avoided by using a column-switching technique [8–13], which allows on-line sample clean-up with no extraction step.

An automated HPLC method has been developed using a column-switching technique for the determination of rifapentine in serum.

EXPERIMENTAL

Materials and reagents

Rifapentine was synthesized by the Medicinal Chemistry Department, Korea Research Institute of Chemical Technology (Daejeon, South Korea). Other drugs such as rifampin, *p*-aminosalicylic acid, isonicotinic acid and acetaminophen were obtained from Sigma (St. Louis, MO, USA).

Standard solutions of rifapentine and rifampin were prepared by dissolving each compound in methanol and diluting to the appropriate concentrations with 1% ascorbic acid solution.

Serum standards in the range 0.1–50 μg/ml were prepared by adding aliquots of rifapentine standard solutions to serum samples.

Chromatographic system

The HPLC system consisted of two Spectra-Physics Model SP 8800 pumps (Santa Clara, CA, USA), a Rheodyne 7125 injector (Cotati, CA, USA), a ten-port multifunction valve (Valco, Houston, TX, USA) and a Spectra-Physics SP 8450 UV–VIS detector. Data handling was performed by a SP 4270 computing integrator (Spectra-Physics).

The instrument arrangement for the ten-port column-switching system was exactly as described by Lee *et al.* [12].

The precolumn (40 × 20 mm I.D.) was dry-packed with Corasil RP C_{18} (37–50 μm, Waters Assoc., Milford, MA, USA). A guard column (20 × 4.6 mm I.D.) was filled by tapping with LiChrosorb RP-8 (25–40 μm, Merck) and the analytical column was μBondapak C_{18} (300 × 3.9 mm I.D., 10 μm; Waters Assoc.). The washing solvent was 0.05 *M* phosphate buffer (pH 7.0) and the mobile phase was acetonitrile–tetrahydrofuran–0.05 *M* phosphate buffer (pH 7.0) (42:5:53, v/v/v) at a flow-rate of 1.0 ml/min. The column temperature was ambient and the wavelength of the detector was 332 nm.

Analytical procedure

A 100-μl sample of the spiked serum or serum from dogs and 300 μl of internal standard in 1% ascorbic acid solution (10 μg/ml rifampin) were mixed and 100 μl of the mixture were injected onto the column. The prepared samples were stored at 4°C before injection.

The sequence of sample analysis included the following three steps and required about 25 min for completion.

Step I (0–5 min). The diluted serum sample was injected onto the precolumn. Polar interfering serum components were washed out to waste. The guard column and analytical column were equilibrated with the mobile phase.

Step II (6–9 min). The washing solvent passed directly to waste. The retained components were eluted from the precolumn to the guard column/analytical column in the back-flush mode by the mobile phase.

Step III (10–25 min). The eluted drugs were separated in the analytical column. The precol-

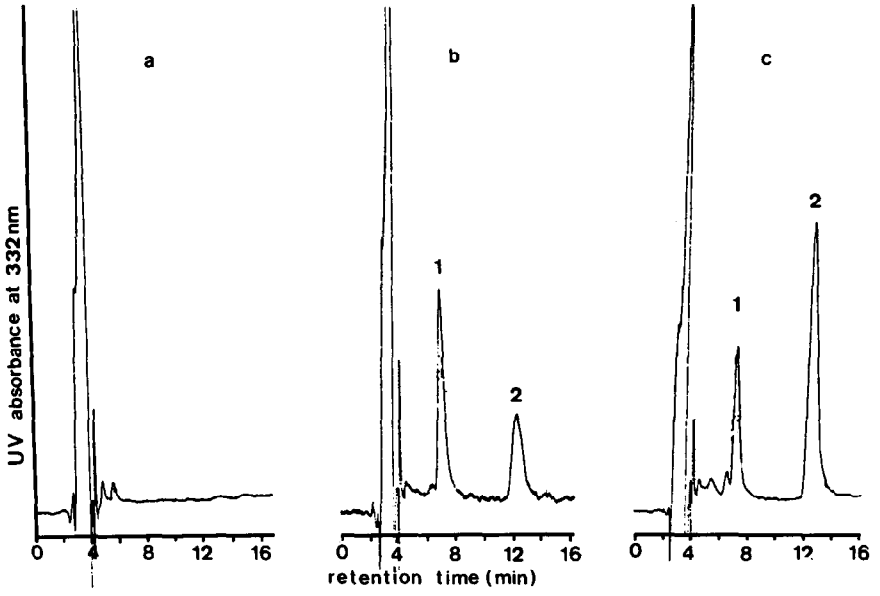

Fig. 1. Chromatograms of (a) blank serum from dogs, (b) blank serum spiked with rifapentine (5 μg/ml) and (c) serum sample from a dog 3 h after the oral administration of rifapentine (10 mg/kg). Peaks: 1 = rifampin (internal standard); 2 = rifapentine.

umn was meanwhile re-equilibrated with the washing solvent for the next injection.

RESULTS AND DISCUSSION

Chromatography and column switching

A typical chromatogram of the separation of rifapentine and rifampin is shown in Fig. 1. As shown in Fig. 1a, there were no interfering peaks at the retention times of rifapentine (12.6 min) and rifampin (7.2 min).

Interferences due to the presence of other drugs such as *p*-aminosalicyclic acid, isonicotinic acid, caffeine, acetaminophen, acetylsalicylic acid, ibuprofen, theophylline, multivitamins (A, C, B_1, B_2, B_6), cefuroxime and ciprofloxacin were ruled out after analysing for these compounds, at the concentrations normally present in serum.

Corasil RP C_{18} (37–50 μm), a non-polar alkyl-bonded phase adsorbent, is a suitable precolumn packing material because of its strong adsorptivity for rifapentine at pH 7.0, its stability at pH 1–7 and its ready availability.

Linearity and limit of detection

The correlation of peak-area ratios with the concentration of rifapentine in serum was linear in the range 0.1–50 μg/ml. The correlation coefficient was greater than 0.999.

The detection limit was determined as the concentration of compound giving a signal-to-noise ratio greater than 3:1. The limit of detection of rifapentine in serum samples was 0.1 μg/ml after the injection of 100 μl of diluted serum (equivalent to 25 μl of serum).

Recovery

The recovery of drug from serum was determined by the analysis of a fixed amount of drug in serum samples, followed by a replicate injection of the same amount of a standard in 10 μl of buffer directly onto the analytical column to give the 100% value. The mean absolute recovery was 91.0 ± 4.1%.

Reproducibility

The precision [defined as the coefficient of variation (C.V.) of replicate analyses] and the accuracy (defined as the deviation between the concentration added and found) of the assay for rifapentine were evaluated over the serum concentration range 1–50 μg/ml. The results are shown in Table I. The C.V. varied from 2.8 to 4.8% over the serum concentration range 1–50 μg/ml and the measured concentration of rifapentine ranged from 96.0 to 100.2% of the added amount in the spiked serum samples.

Application of the method to biological samples

The method was successfully applied to the

TABLE I

REPRODUCIBILITY OF RIFAPENTINE ASSAY IN SERUM SAMPLES (n = 5)

Concentration (μg/ml)		C.V. (%)	Difference between concentration added and found (%)
Added	Found		
Intra-assay reproducibility			
1.0	0.99	4.5	−2.0
10.0	9.6	4.3	−4.0
20.0	19.4	3.6	−3.0
50.0	49.3	2.8	−1.4
Inter-assay reproducibility			
1.0	1.0	4.8	0.0
10.0	9.8	4.7	−2.0
20.0	19.3	3.7	−3.5
50.0	50.1	2.8	0.2

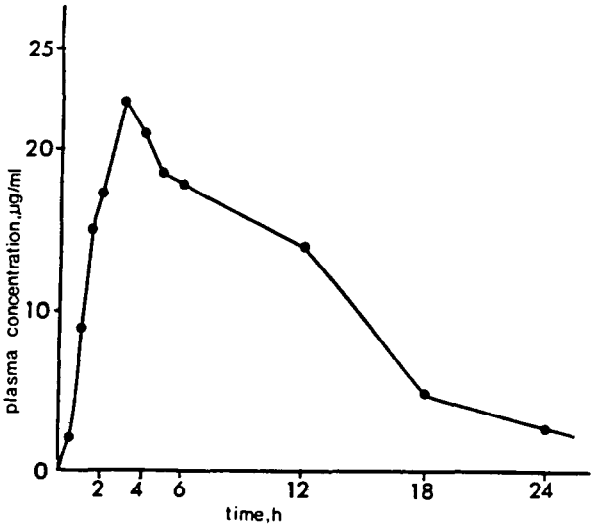

Fig. 2. Concentration *versus* time curve of serum samples from a dog after the oral administration of rifapentine (10 mg/kg).

analysis of about 300 serum samples from Beagle dogs. The chromatogram of a serum sample from a dog after the oral administration of rifapentine (10 mg/kg) is shown in Fig. 1c. Fig. 2 shows a serum concentration *versus* time plot from a dog after the oral administration of rifapentine (10 mg/kg).

CONCLUSIONS

An automated HPLC method with the direct injection of diluted serum samples was developed, using a column-switching technique, for the determination of rifapentine. This method was shown to be more rapid, sensitive and reproducible than previously reported methods.

REFERENCES

1 R. Cricchio, V. Arioli and G. C. Lancini, *Farmaco, Ed. Sci.*, 30 (1975) 605.
2 H. Baba and Y. Azuma, *Kekkaku*, 51 (1976) 1.
3 V. Arioli, M. Berti, G. Carniti, E. Randisi, E. Rossi and R. Scotti, *J. Antibiot.*, 34 (1981) 1026.
4 A. T. Birmingham, A. J. Coleman, M. Le Horme, B. K. Park, N. J. Pearson, A. H. Short and P. J. Southgate, *Br. J. Clin. Pharmacol.*, 6 (1978) 455.
5 A. Assandri, B. Ratti and T. Cristina, *J. Antibiot.*, 37 (1984) 1066.
6 B. Ratti, A. Toselli and R. R. Parenti, *Farmaco, Ed. Prat.*, 36 (1981) 319.
7 T. Z. Zeng, Z. Chen, M. Z. Liang and Y. Huang, *Yaowu Fenxi Zazhi*, 7 (1987) 195.
8 W. Voelter, T. Kronbach, K. Zech and R. Huber, *J. Chromatogr.*, 239 (1982) 475.
9 R. Wyss and F. Bucheli, *J. Chromatogr.*, 424 (1988) 303.
10 H. S. Lee, E. J. Kim, O. P. Zee and Y. J. Lee, *Arch. Pharm. Res.*, 12 (1989) 108.
11 H. S. Lee, E. J. Kim, O. P. Zee and Y. J. Lee, *Arch. Pharm. (Weinheim)*, 322 (1989) 801.
12 H. S. Lee, O. P. Zee and K. I. Kwon, *J. Chromatogr.*, 528 (1990) 425.
13 Y. J. Lee and H. S. Lee, *Chromatographia*, 30 (1990) 80.