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Simultaneous determination of cefamandole and cefamandole nafate in human plasma and urine by high-performance liquid chromatography with column switching

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SUMMARY

A high-performance liquid chromatographic method with column switching has been developed for the simultaneous determination of cefamandole and cefamandole nafate in plasma and urine. The plasma and urine samples were injected onto a precolumn packed with Corasil RP C_{18} (37–50 μm) after simple dilution with an internal standard solution in 0.05 *M* phosphoric acid. Polar plasma and urine components were washed out using 0.05 *M* phosphoric acid. After valve switching, the concentrated drugs were desorbed in back-flush mode and separated by a reversed-phase C_8 column with methanol–5 m*M* tetrabutylammonium bromide (45.55, v/v) as the mobile phase. The method showed excellent precision with good sensitivity and speed, and a detection limit of 0.5 μg/ml. The total analysis time per sample was less than 30 min, and the mean coefficients of variation for intra- and inter-assay were both less than 4.9%. The method has been successfully applied to plasma and urine samples for human volunteers after intravenous injection of cefamandole nafate.

INTRODUCTION

Cefamandole (Fig. 1) is a semisynthetic cephalosporin antibiotic with broad spectrum of antimicrobial activity [1]. Cefamandole nafate (Fig. 1) is the so-

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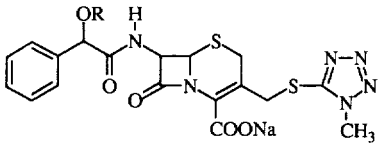

R = H Cefamandole
R = CHO Cefamandole nafate

Fig. 1. Structures of cefamandole and cefamandole nafate.

dium salt of 7-D-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid formyl ester, and it is the preferred form of the product for reasons of crystallinity and stability. Upon reconstitution, cefamandole nafate hydrolyses to cefamandole and formate ion, and there is a marked effect of pH on the hydrolysis rate: hydrolysis proceeds more rapidly at higher pH. At physiological temperature (37°C) and pH (7.4), the hydrolysis proceeds with a half-life of 28.5 min [2].

High-performance liquid chromatographic (HPLC) methods have been developed previously for the determination of cefamandole in biological fluids [2–10]. One of these papers [2] describes the simultaneous determination of cefamandole and cefamandole nafate, but it is not suitable to quantify routinely the drugs in plasma and urine simultaneously. In addition, all these methods are time-consuming and tedious because of the deproteinization or liquid–liquid extraction required for sample clean-up.

Therefore, an automated HPLC method with direct injection of diluted plasma or urine samples was developed using a column-switching technique for on-line sample clean-up [11–20]. This enabled the simultaneous determination of cefamandole and cefamandole nafate without an extraction procedure.

EXPERIMENTAL

Materials and reagents

Cefamandole and cefamandole nafate were received from Samchunri Pharmaceutical (Seoul, Korea). Cephalothin was obtained from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade, including *n*-tetrabutylammonium bromide from Serva (Heidelberg, F.R.G.).

Standard solutions of cefamandole, cefamandole nafate and cephalothin were prepared by dissolving each compound in water and diluting to the appropriate concentrations with 0.05 *M* phosphoric acid.

Plasma and urine samples

Blood samples were collected in glass test-tubes (Vacutainer) containing heparin, immediately before and at appropriate times after drug administra-

tion. The blood samples were cooled in ice, and a 1.0-ml aliquot was removed immediately and placed in a tube containing 0.1 ml of 2 *M* sodium acetate buffer (pH 5.0) and 0.1 ml of a 0.05% solution of tri-*o*-tolyl phosphate (TOTP) in methanol. TOTP was added as a carboxyl esterase inhibitor for the prevention of possible hydrolysis due to serum esterase activity [2]. After centrifugation, the plasma was removed and 0.01 ml of glacial acetic acid was added. The samples were frozen at −20°C until analysis.

Urine samples were collected, and 0.5 ml of 2 *M* sodium acetate buffer (pH 3.0) was added to a 1.5-ml aliquot of urine. The urine samples were also stored at −20°C until analysis.

Chromatographic system

A schematic representation of the ten-port column-switching system is given in Fig. 2. Pump 1 (M 501 pump, Waters Assoc., Milford, MA, U.S.A.) delivered washing solvent at a flow-rate of 1.0 ml/min. Aliquots (100 μl) of diluted plasma or urine were injected by a ten-port multifunction valve (Valco, Houston, TX, U.S.A.) onto the precolumn. Pump 2 (SP 8800 pump, Spectra Physics, Santa Clara, CA, U.S.A.) delivered mobile phase at a flow-rate of 1.0 ml/min for the elution of the adsorbed components from the precolumn in the back-flush mode onto the analytical column. An injector (Rheodyne 7125 with a 10-μl loop, Cotati, CA, U.S.A.), located between pump 2 and the multifunction valve, was used for direct injection onto the analytical column. Detection of the eluted compounds was carried out at 270 nm with a UV detector (SP 8450 UV–VIS detector, Spectra Physics), and data-handling was performed by means of a computing integrator (SP 4270, Spectra Physics).

The precolumn (40 mm×2.0 mm I.D.) was filled by gentle tapping with Corasil RP C_{18} (37–50 μm, Waters Assoc.) and was changed after injection of every 60 samples. A guard column (40 mm×4.6 mm I.D.) was filled by tapping with Co:Pell ODS (37–53 μm, Whatman, Clifton, NJ, U.S.A.) and the analytical column was a 250 mm×4 mm I.D. column prepacked with 10-μm LiChrosorb RP-8 (E. Merck).

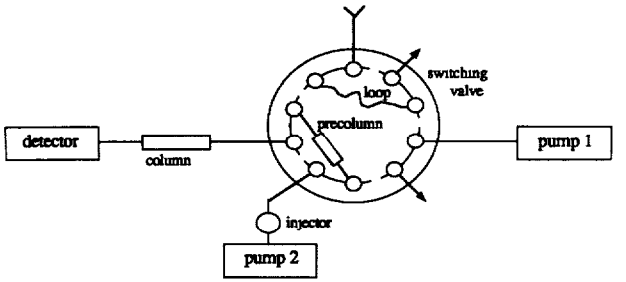

Fig. 2. Schematic diagram of ten-port switching system. The load setting is shown by the dashed lines, and the injection setting by the continuous lines.

The washing solvent was 0.05 *M* phosphoric acid, and the mobile phase was 5 m*M* tetrabutylammonium bromide in water containing methanol (55:45, v/v).

Analytical procedure

A 200-μl sample of the spiked plasma or patient plasma and 200 μl of internal standard in 0.05 *M* phosphoric acid (20 μg/ml cephalothin) were mixed, and 100 μl of the mixture were injected. Urine samples were diluted with 0.05 *M* phosphoric acid to provide concentrations within the range of the calibration curve, and the final concentration of internal standard was 20 μg/ml. The prepared samples were kept at 4°C before injection.

The sequence of the sample analysis included the following three steps and required about 30 min.

Step I (0–10 min). The diluted plasma or urine sample was injected onto the precolumn. Polar interfering plasma or urine components were washed out to waste. The guard column and analytical column were equilibrated with the mobile phase.

Step II (11–14 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 (15–30 min). The eluted drugs were separated in the analytical column. Meanwhile the precolumn was re-equilibrated with the washing solvent for the next injection.

RESULTS AND DISCUSSION

Chromatography

The chromatographic conditions for cefamandole on reversed-phase material have been reported previously [2–10]. Also, the simultaneous determination of cefamandole and cefamandole nafate has been reported by Wold et al. [2], but the resolution was poor and the lifetime of the column was short.

In this study, the addition of an ion-pair reagent (tetrabutylammonium bromide) permitted the simultaneous determination of cefamandole and cefamandole nafate in plasma and urine with better resolution. Typical chromatograms illustrating the separation of cefamandole and cefamandole nafate are shown in Figs. 3 and 4. The resolution values were as follows: R_s (cefamandole–cephalothin) = 3.26; R_s (cephalothin–cefamandole nafate) = 1.70. As shown in Fig. 3a and 4a, there were no interfering peaks at the retention times of cefamandole and cefamandole nafate. The lifetime of the analytical column was well over six months under intensive use.

Possible interference with other antibiotics and caffeine was investigated, and the relative retention times are summarized in Table I. This demonstrates

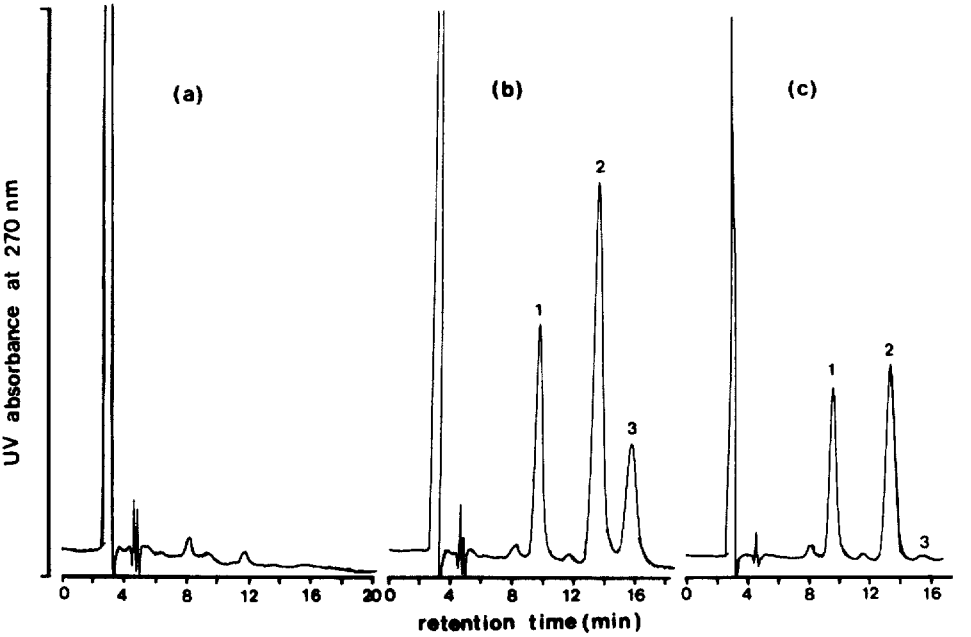

Fig. 3. Chromatograms of (a) human blank plasma, (b) blank plasma spiked with cefamandole and cefamandole nafate (10 μg/ml each) and (c) plasma of a volunteer 90 min after an intravenous injection of 1 g of cefamandole nafate. Peaks: 1 = cefamandole; 2 = cephalothin (internal standard); 3 = cefamandole nafate.

that none of the tested substances should interfere with the determination of cefamandole and cefamandole nafate.

Column-switching procedure

The characteristic ring structure and ionizable carboxylic acid group of cefamandole and cefamandole nafate lead to stronger retention on a non-polar alkyl bonded-phase column. Corasil RP C_{18} (37–50 μm), a non-polar octadecylsilane bonded-phase adsorbent, is a suitable precolumn packing because of its strong adsorptivity for cefamandole and cefamandole nafate at pH < 4 and stability at pH 1–7.

The choice of 0.05 *M* phosphoric acid for washing solvent was based on the following considerations: at acidic pH (pH < 2), the charges of the amino acid groups on albumin change and cefamandole and cefamandole nafate are present in non-ionic form. Consequently, although the majority of the plasma and urine components are relatively unretained on the Corasil RP C_{18} precolumn, cefamandole and cefamandole nafate exhibit strong adsorption on Corasil RP C_{18} from this solvent.

The clean-up process, to obtain sharp peaks and clean chromatograms, was completed in less than 10 min by washing the precolumn at a flow-rate of 1.0 ml/min with 0.05 *M* phosphoric acid solution.

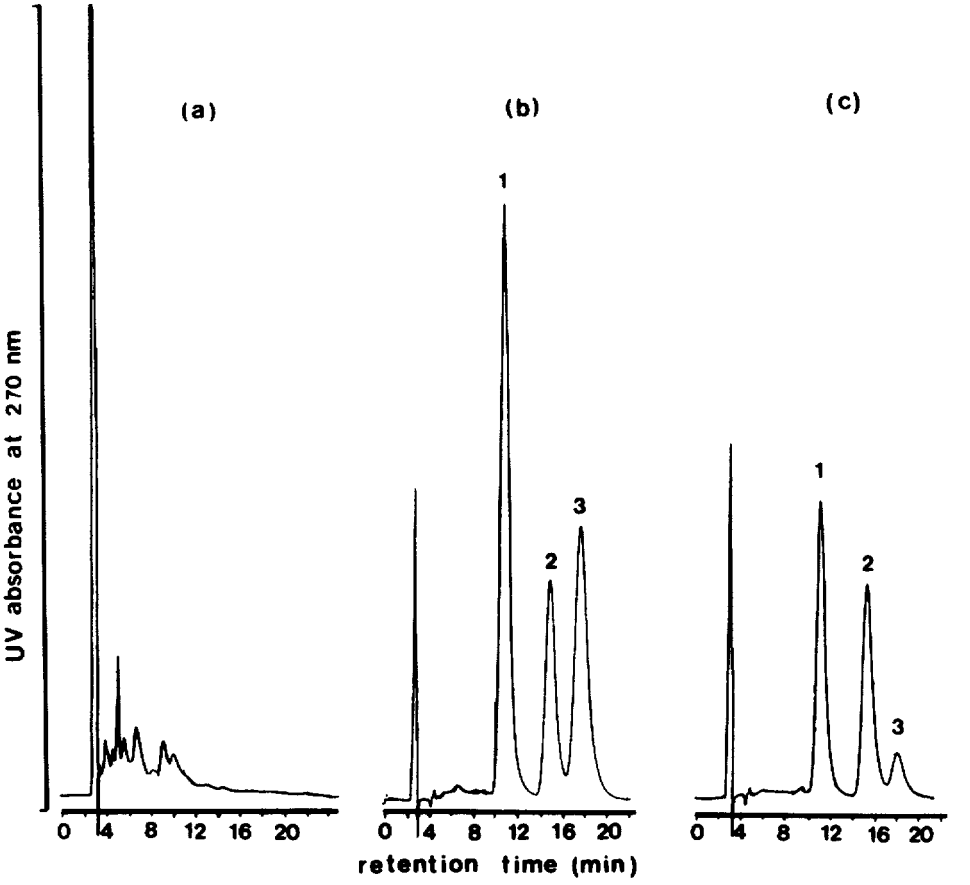

Fig. 4. Chromatograms of (a) human blank urine, (b) blank urine spiked with cefamandole and cefamandole nafate (50 μg/ml each) and (c) urine of a volunteer 1 h after an intravenous injection of 1 g of cefamandole nafate. Peaks: 1 = cefamandole; 2 = cephalothin; 3 = cefamandole nafate.

Linearity and detectability

The correlation of peak-area ratios with the concentrations of cefamandole and cefamandole nafate in plasma or urine was linear in the range 0.5–100 μg/ml. The correlation coefficients were better than 0.999.

Detection limits were determined as the concentration of compound giving a signal-to-noise ratio greater than 3:1. The limit of detection of cefamandole and cefamandole nafate in plasma samples was 0.5 μg/ml after an injection of 100 μl of diluted plasma (equivalent to 50 μl of plasma).

Recovery

The recovery of drugs from plasma or urine was determined by the analysis of fixed amount of drugs in plasma or urine samples, followed by replicate

TABLE I

RELATIVE RETENTION TIMES OF OTHER ANTIBIOTICS AND SOME COMMON DRUGS

Substance	Relative retention time
Cefotiam	0.30
Cephalexin	0.34
Cephaloridine	0.39
Caffeine	0.54
Cefotaxime	0.59
Cefuroxime	0.64
Cefazolin	0.64
Cefoperazone	0.70
Cefoxitin	0.76
Cefamandole	1.00
Cephalothin	1.39
Cefamandole nafate	1.64
Phenobarbital	N.D.[a]

[a]Not detectable in 30 min.

injection of the same amount of a standard in 10 μl buffer directly onto the analytical column, providing the 100% value. Mean recoveries of cefamandole and cefamandole nafate were 91.5 and 90.9% in plasma and 94.2 and 91.9% in urine, respectively.

Reproducibility

The precision [defined as the coefficient of variation (C.V.) of replicate analysis] and the accuracy (defined as the deviation between added and found concentration) of the assay for cefamandole and cefamandole nafate were evaluated over the plasma concentration range 1–100 μg/ml. The results are shown in Table II. The C.V. varied from 1.7 to 4.9% over the plasma concentration range 1–100 μg/ml and the measured concentration of cefamandole and cefamandole nafate ranged from 96.0 to 103.2% of the added amount in the spiked plasma samples.

Application of the method to biological samples

The present method was successfully applied to the analysis of more than 800 plasma and urine samples from eight human volunteers. The plasma and urine chromatograms of a human volunteer after intravenous injection (1 g) of cefamandole nafate are shown in Figs. 3c and 4c, respectively. Fig. 5 shows a plasma concentration versus time plot from a human volunteer after the intravenous injection of 1 g of cefamandole nafate.

TABLE II

REPRODUCIBILITY OF CEFAMANDOLE (C) AND CEFAMANDOLE NAFATE (CN) ASSAY IN PLASMA SAMPLES ($n=5$)

Concentration (μg/ml)			C.V. (%)	
Added	Found		C	CN
	C	CN		
Within-day				
1.0	0.96	0.97	3.6	4.5
10.0	9.7	9.8	2.4	3.5
25.0	25.5	25.3	1.7	3.3
50.0	48.7	48.9	1.9	4.0
100.0	101.2	98.5	3.7	4.3
Day-to-day				
1.0	1.0	0.98	4.3	4.9
10.0	9.6	9.7	3.5	4.1
25.0	25.8	24.3	1.8	3.5
50.0	49.0	50.3	3.2	4.4
100.0	100.8	98.4	3.9	4.6

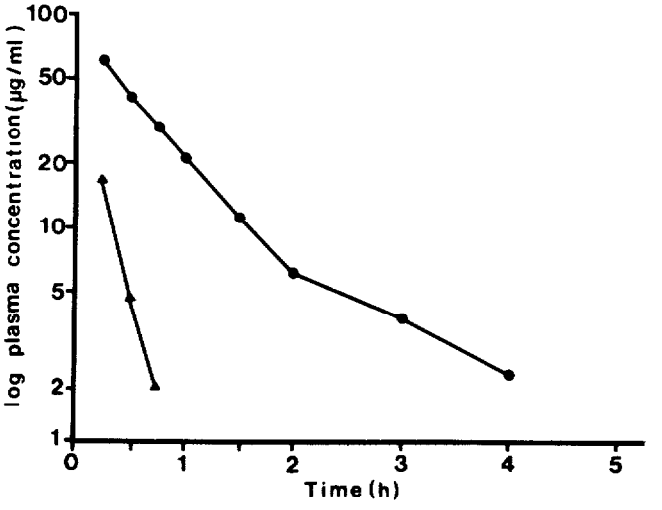

Fig. 5. Plasma concentrations versus time curve of a human volunteer after intravenous injection of 1 g of cefamandole nafate. (●) Cefamandole; (▲) cefamandole nafate.

CONCLUSION

An automated HPLC method with direct injection of diluted plasma and urine samples was developed, using the column-switching technique, for the

simultaneous determination of cefamandole and cefamandole nafate. Time-consuming extraction steps were avoided, and therefore hydrolysis of cefamandole nafate to cefamandole could be limited in analysing the plasma and urine samples. This method was shown to be sensitive enough to quantify cefamandole and cefamandole nafate in biological samples after intravenous injection of a clinical dose (Fig. 5).

REFERENCES

1 H.C. Neu, Antimicrob. Agents Chemother., 6 (1974) 117.
2 J.S. Wold, R.R. Toost, H.R. Black and R.S. Griffith, J. Infect. Dis., 137 (Suppl.) (1978) 17.
3 N.S. Aziz, J.G. Gambertoglio, E.T. Lin, H. Grausz and L.Z. Benet, J. Pharmacokin. Biopharm., 6 (1978) 153.
4 A.M. Brisson and J.B. Fourtillan, J. Chromatogr., 223 (1981) 393.
5 M.C. Rouan, F. Abadie, A. Leclerc and F. Juge, J. Chromatogr., 275 (1983) 133.
6 L.A. Danzer, Clin. Chem., 29 (1983) 856.
7 R.E. Bawdon, K.J. Leveno, J.G. Quirk, F.G. Cunningham and S.P. Guss, J. Liq. Chromatogr., 6 (1983) 2747.
8 T. Annesley, K. Wilkerson, K. Matz and D. Giacherio, Clin. Chem., 30 (1984) 908.
9 S.A. Signs, T.M. File and J.S. Tan, Antimicrob. Agents Chemother., 26 (1984) 652.
10 M. Bliss and M. Mayersohn, Clin. Chem., 32 (1986) 197.
11 W. Roth, K. Beschke, R. Janch, A. Zimmer and F.W. Koss, J. Chromatogr., 222 (1981) 13.
12 J.B. Lecaillon, M.C. Rouan, C. Souppart, N. Febvre and F. Juge, J. Chromatogr., 228 (1982) 257.
13 R.A. Hux, H.Y. Mohammed and F.F. Cantwell, Anal. Chem., 54 (1982) 113.
14 W. Voelter, T. Kronbach, K. Zech and R. Huber, J. Chromatogr., 239 (1982) 475.
15 M.W.F. Nielen, R.C.A. Koordes, R.W. Frei and U.A.Th. Brinkman, J. Chromatogr., 330 (1985) 113.
16 W.S. Powell, Anal. Biochem., 164 (1987) 117.
17 D. Dadgar and A. Power, J. Chromatogr., 416 (1987) 99.
18 R. Wyss and F. Bucheli, J. Chromatogr., 424 (1988) 303.
19 H.S. Lee, E.J. Kim, O.P. Zee and Y.J. Lee, Arch. Pharm. (Weinheim), 322 (1989) 801.
20 H.S. Lee, E.J. Kim, O.P. Zee and Y.J. Lee, Arch. Pharm. Res., 12 (1989) 108.