Journal of Chromatography, 495 (1989) 354–357
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4890

Letter to the Editor

Determination of ofloxacin in bronchoalveolar lavage fluid by high-performance liquid chromatography and fluorimetric detection

Sir,

Ofloxacin is one of the fluorinated quinolones structurally related to nalidixic acid The compound exhibits potent antibacterial activity against a variety of gram-positive and gram-negative pathogens, its bactericidal action is based on its anti-DNA gyrase activity [1] Owing to its favourable antibacterial and pharmacokinetic profiles, ofloxacin has been used for treatment of respiratory tract infections as well as urinary tract infections [1,2] The prime objective of antibacterial chemotherapy is to help the host to eliminate invading organisms by providing an optimal amount of the drug in infected tissues In this connection, the distribution of ofloxacin to the human lung has been studied in order to determine the optimal regimen for respiratory tract infections [3] However, the penetrability of ofloxacin into the lungs has not been fully clarified, because of the lack of methods for determining low drug concentrations in specimens such as bronchoalveolar lavage fluid, in which the drug is extensively diluted. We have developed a highly sensitive high-performance liquid chromatographic (HPLC) method to determine the concentration of ofloxacin in bronchoalveolar lavage fluid Notarianni and Jones [4] and Le Coguic et al [5] have also proposed sensitive assays for serum and urine samples

Ofloxacin and 9-fluoro-2,3-dihydro-3-methyl-10-(1-imidazolyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4] benzoxazine-6-carboxylic acid (internal standard, I.S.) were synthesized at the Research Institute of Daiichi Seiyaku (Tokyo, Japan). Tetrahydrofuran was of HPLC grade and obtained from Merck (Darmstadt, F.R.G.).

The HPLC system consisted of a SP8800 solvent-delivery system (Spectra-Physics, San Jose, CA, U.S A), a sample injector (Model EIE-005, Senshu Kagaku, Tokyo, Japan) fitted with a 50-μl loop, a guard-filter (0 45 μm pore

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size, Irika Kikai, Kyoto, Japan), a prepacked stainless-steel column (15 cm × 4 6 mm I D) packed with 5-μm particle size octadecylsilica (TSK ODS-80TM, Tosoh, Tokyo, Japan) and a variable-wavelength spectrofluorimeter (Model F1000, Hitachi, Tokyo, Japan) The mobile phase consisted of a mixture of phosphate buffer (the pH of a 0 5%, w/v, solution of potassium dihydrogenphosphate was adjusted to 2 9 by titration with orthophosphoric acid) and tetrahydrofuran (16 1, v/v) The flow-rate was 1 0 ml/min Elution was carried out in isocratic mode. The eluate was monitored fluorimetrically (290 nm excitation and 460 nm emission). The retention times for ofloxacin and the internal standard were 12 and 8 min, respectively

To 1 0 ml of bronchoalveolar lavage fluid in a 12-ml glass centrifuge tube, 20 μl of a solution of internal standard (30 μg/ml) and 1 0 ml of 0 1 *M* phosphate buffer (pH 7 0) were added and mixed on a vortex mixer Then, 5 ml of chloroform were added and the tube was sealed with a glass stopper For extraction, the tube was laid down and shaken horizontally at a rate of 2 strokes/s for 10 min. After centrifugation at 1500 *g* for 10 min, the chloroform layer was transferred to another glass tube and evaporated to dryness under a gentle stream of nitrogen at 40°C The residue was reconstituted in 0 4 ml of mobile phase, and a 20-μl portion was injected into the column

Standard samples were prepared by dissolving ofloxacin in control bronchoalveolar lavage fluid or the 0 1 *M* phosphate buffer to final concentrations in the range 0.48–95 1 ng/ml. A calibration curve was obtained by plotting the peak-height ratios against the concentrations of ofloxacin in the standard samples. The calibration line was calculated by the weighted least-squares linear

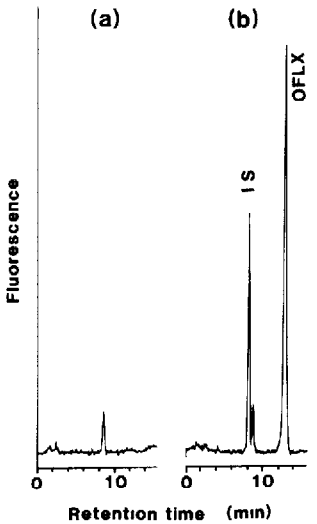

Fig 1 Chromatograms of the extracts of bronchoalveolar lavage fluids (a) control, (b) spiked with ofloxacin Peaks OFLX = ofloxacin, I S = internal standard

TABLE I

ACCURACY AND PRECISION OF THE PROPOSED METHOD FOR DETERMINATION OF OFLOXACIN IN BRONCHOALVEOLAR LAVAGE FLUID

Ofloxacin (ng/ml)		Accuracy [(found/added) × 100] (%)	Precision, C V (%)
Added	Found (mean ± S D)		
Intra-assay (n = 10)			
0 95	0 97 ± 0 03	101 6	2 9
9 51	9 32 ± 0 26	98 0	2 8
47 6	47 71 ± 1 20	100 3	2 5
95 1	95 11 ± 3 87	100 0	4 1
Inter-assay (n = 5)			
0 95	1 11 ± 0 11	117 2	9 9
9 51	9 82 ± 0 66	103 2	6 7
47 6	48 40 ± 2 90	101 8	6 0
95 1	95 66 ± 4 21	100 6	4 4

regression method, and the weight was a reciprocal of the concentration ($1/x$)

To establish a highly sensitive method for ofloxacin in bronchoalveolar lavage fluid, we tried to modify the HPLC–UV method of Ichihara et al [2] by the use of fluorimetric detection to improve the detectability, since ofloxacin is a strongly fluorogenic compound However, a broad interfering peak was detected and it could not be well resolved with the previous mobile phase, which contained acetonitrile or methanol When the acetonitrile or methanol was replaced with tetrahydrofuran, the substance in control bronchoalveolar lavage fluid eluted as a sharp peak and did not interfere with the determination of ofloxacin (Fig 1). Although the substance was not identified, it might come from the drugs used in bronchoalveolar lavage treatment.

The absolute calibration curve deviated upward from the predicted straight line in the lower concentration range This phenomenon was found to be due to a small amount of the compound adsorbed on the ground-glass surface of the manual syringe This type of carry-over was also reported in the analysis of ciprofloxacin by Myers and Blumer [6], and they used distilled water or 0 89% saline as washing solvent for the syringe to eliminate carry-over However, we would recommend a mixture of 0 02 *M* orthophosphoric acid and an organic solvent, such as acetonitrile or tetrahydrofuran (1 1, v/v).

The internal standard is 16 times less fluorogenic than ofloxacin. This property makes the compound useful not only as a carrier that could prevent adsorption of the test drug on the glass surface, but also as an internal standard In fact, in this study, the internal standard and ofloxacin could be detected

within the same range of sensitivity, even though the internal standard was used at a concentration 60 times that of ofloxacin.

Calibration curves were linear over the range 0 5–100 ng/ml The control bronchoalveolar lavage fluid could be substituted with 0 1 *M* phosphate buffer without any significant change of the linearity and the slope. The intra-assay coefficients of variation (C.V.) were below 5%, even at concentrations less than 1 ng/ml (Table I), whereas Le Coguic et al [5] did not report values at such a low level. Absolute recovery was more than 95%, and this shows that chloroform is a more suitable extraction solvent than dichloromethane, which was used by Le Coguic et al [5]

The method established here offers another sensitive means to determine ofloxacin at the ng/ml level, and could be generally applied to a variety of clinical specimens, in which the drug concentrations are very low

ACKNOWLEDGEMENT

We are grateful to Dr. Masahiro Miyai, Department of Medicine, Okayama City Hospital, Okayama, Japan, for offering us human bronchoalveolar lavage fluid specimens

Research Institute, Daiichi Seiyaku Co , Ltd ,
16-13, Kitakasai 1-Chome, Edogawa-ku,
Tokyo 134 (Japan)

KYUICHI MATSUBAYASHI*
TSUTOMU UNE
YASUAKI OSADA

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(First received January 18th, 1989, revised manuscript received May 30th, 1989)