Journal of Chromatography A, 653 (1993) 135–137
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 442

Short Communication

High-performance liquid chromatographic preparation of oxybutynin enantiomers on a chiral stationary phase☆

Etsuko Miyamoto*, Yoko Demizu, Yoshifumi Murata, Yutaka Yamada and Susumu Kawashima

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-11 (Japan)

Hitoshi Kontani and Takeshi Sakai

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-11 (Japan)

(First received May 25th, 1993; revised manuscript received July 14th, 1993)

ABSTRACT

A method for the preparation of oxybutynin enantiomers by high-performance liquid chromatography (HPLC) on a chiral stationary phase (Chiralpak AD) was developed. Enantiomers were separated directly and rapidly without the need for any derivatization. Good optical resolution was obtained, with separation and resolution factors of 1.43 and 1.28, respectively. This method would be a useful alternative to synthetic preparation of the enantiomers.

INTRODUCTION

Oxybutynin (Fig. 1) is used as a racemate for the treatment of urinary incontinence due to detrusor instability. It has two enantiomers, which show different pharmacological properties [1,2], as well as many pharmaceuticals [3], although pharmacokinetic studies of oxybutynin have been carried out using the racemate [4,5]. Simple preparation and determination of the two enantiomers would be expected to facilitate further investigation of their properties. Chiral stationary phases for high-performance liquid chromatography (HPLC) have been introduced for use in the separation of many racemates [6].

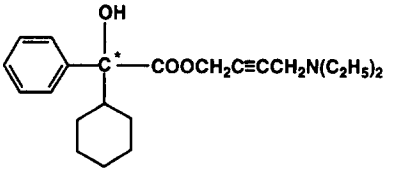

Fig. 1. Structure of oxybutynin.

* Corresponding author.

☆ Presented in part at the *113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, March 1993.*

0021-9673/93/$06.00

However, HPLC resolution of oxybutynin enantiomers has not been reported.

In this study, direct preparation of oxybutynin enantiomers was attempted using carbamate derivatives of amylose coated on silica gel as a chiral stationary phase for HPLC.

EXPERIMENTAL

Materials

Oxybutynin chloride (Sigma, St. Louis, MO, USA) and all other chemicals were of reagent grade obtained commercially and used without further purification.

Preparation of oxybutynin free base

Oxybutynin free base was prepared as follows. Oxybutynin chloride, 1 g, was dissolved in 100 ml of phosphate buffer (pH 8) and extracted twice with 100 ml of *n*-hexane. The organic phase was evaporated under reduced pressure at 30°C. The recovery of oxybutynin as the free base was more than 90%. It was dissolved in the HPLC eluent to give a concentration of 5 mg ml^{-1} and filtered through a 0.45-μm membrane filter (AcroLC, Gelman Sciences Japan, Tokyo, Japan) before injection.

Apparatus and procedure

The liquid chromatograph was equipped with a pump (Model 510, Waters, Milford, MA, USA), an absorbance detector (254 nm, Model 440, Waters), a Model C-R1B integrator (Shimadzu, Kyoto, Japan) and a Rheodyne 7125 injector valve with a 100-μl sample loading loop. The separation was performed on a Chiralpak AD column (25 × 1 cm I.D., 10 μm particle size, Daicel, Tokyo, Japan) connected to a precolumn (Chiralpak AD, 5 × 1 cm I.D.) thermostated by a column oven (L-5030, Hitachi, Tokyo, Japan) at 25.0°C and eluted with *n*-hexane–2-propanol (90:10, v/v) at a flow-rate of 1.0 ml min^{-1}. The sample volume injected was 100 μl for each 20-min period. The eluates of each peak were collected and evaporated under reduced pressure at 30°C. The circular dichroism (CD) spectrum of each sample ($1.0 \cdot 10^{-3}$ mol l^{-1} in eluent) was measured from 210 to 300 nm (Jasco-J-500C spectropolarimeter, Japan Spectroscopic, Tokyo, Japan). The concentration as total oxybutynin was then determined [7]. The dead time of the column was estimated using toluene.

RESULTS AND DISCUSSION

Fig. 2 shows a typical chromatogram of the resolution of oxybutynin racemate. The racemate used contained the same amount of each enantiomer, as estimated from the ratio of their peak areas, 0.995 ± 0.003. The HPLC resolution, capacity factor (k'), separation factor (α) and resolution factor (R_s) were calculated as 0.64 (k'_1) and 0.91 (k'_2), 1.43 and 1.28, respectively. The sample for each enantiomer was analysed by HPLC, and each was confirmed to be a single

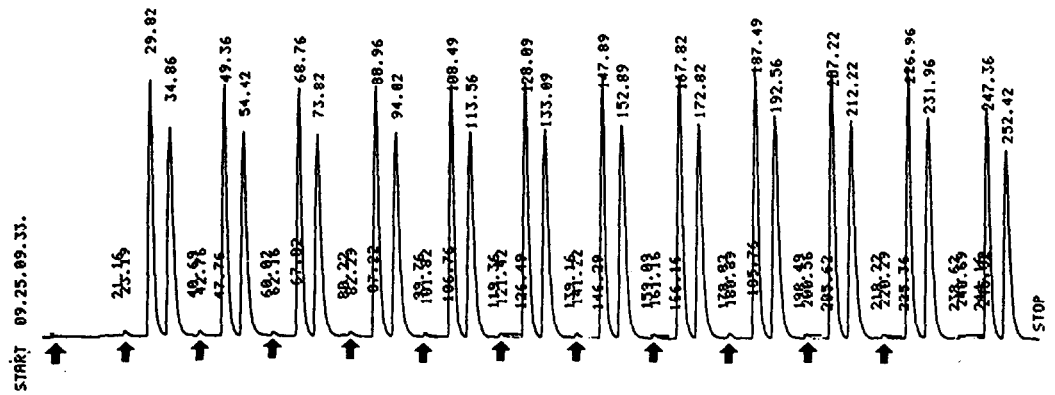

Fig. 2. Chromatographic resolution of oxybutynin racemate injected 100 μl (5 mg ml^{-1}) for each 20-min period. Values at peaks indicate retention times in min.

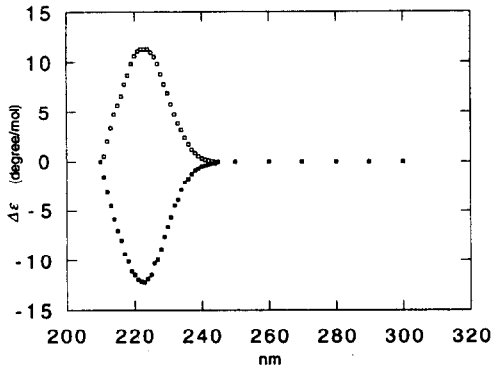

Fig. 3. CD spectra of oxybutynin enantiomers in *n*-hexane–2-propanol (90:10). ■ = Front peak; □ = second peak.

peak. As shown in Fig. 3, the CD spectra of the enantiomers were characterized by a Cotton effect at about 220 nm ($\Delta\varepsilon = -12.0$ mol l^{-1} cm^{-1} for the front peak, +11.4 mol l^{-1} cm^{-1} for the second peak). Racemization of both enantiomers was not observed in the eluent or in methanol during at least 6 months in a refrigerator at 4°C, and both enantiomers were also stable for 2 days in aqueous solution between pH 1.0 and 7.4 at 37.0°C. The contractile response of isolated rat bladder detrusor muscle to concentrations of $1 \cdot 10^{-6}$, 10^{-5} and 10^{-4} mol l^{-1} was compared by measuring the isomeric contractions. The enantiomer prepared from the second peak showed higher potency than the other peak [9].

The HPLC preparation of oxybutynin enantiomers reported here was simple in comparison with synthetic methods [8]. We are now applying this method for simultaneous quantitative determination of enantiomers in biological fluids in order to investigate the pharmacokinetics of oxybutynin enantiomers.

ACKNOWLEDGEMENTS

This work was supported by a grant-in-aid from Hokuriku University. The authors thank Ms. T. Sato and Ms. H. Horikawa for their excellent technical assistance.

REFERENCES

1 J.F. Kachur, J.S. Peterson, J.P. Carter, W.J. Rzeszotarski, R.C. Hanson and L. Noronha-Blob, *J. Pharmacol. Exp. Ther.*, 247 (1988) 867.
2 L. Noronha-Blob and J.F. Kachur, *J. Pharmacol. Exp. Ther.*, 256 (1988) 562.
3 E.J. Ariëns, *Eur. J. Clin. Pharmacol.*, 26 (1984) 663.
4 J. Douchamps, F. Derennc, A. Stockis, D. Gangji, M. Juvent and A. Herchuelz, *Eur. J. Clin. Pharmacol.*, 35 (1988) 515.
5 K.M. Hughes, J.C.T. Lang, R. Lazare, D. Gordon, S.L. Stanton, J. Malone-Lee and M. Geraint, *Xenobiotica*, 22 (1992) 859.
6 E. Francotte and A. Junker-Buchheit, *J. Chromatogr.*, 576 (1992) 1.
7 E. Miyamoto, S. Kawashima, Y. Murata, Y. Yamada, Y. Demizu, H. Kontani and T. Sakai, *J. Pharm. Sci.* (1993), submitted for publication.
8 R.B. Barlow, F.M. Franks and J.D.M. Pearson, *J. Med. Chem.*, 16 (1973) 439.
9 E. Miyamoto, presented in part at the *113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, March 29–31, 1993*, poster 30PC10-30.