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A New HPLC Technique for the Separation of Methocarbamol Enantiomers*

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Abstract

We have developed a stereoselective high-performance liquid chromatography technique for analytical separation of methocarbamol enantiomers.

Precolumn derivatization was performed at room temperature using (−)-menthylchloroformate as a chiral reagent in the presence of pyridine as catalyst. The resulting diastereomers were separated on two Resolve C18 columns connected in series. The mobile phase was phosphate buffer (pH 7·5)-acetonitrile (50 : 50, v/v) at a flow rate of 1 mL min^{-1}. UV detection was set at 274 nm.

The optimum amount of reagent and the maximum peak intensity of the diastereomers were determined. The resolution of the diastereomers was satisfactory ($\alpha = 1{\cdot}04$) under the conditions used.

Methocarbamol, a carbamate analogue of aryl glycerol ethers (Figure 1), is a centrally active muscle relaxant used in the symptomatic treatment of musculoskeletal disorders (White 1995). The molecule contains an asymmetric carbon, and the racemate is used clinically. Numerous studies have revealed that drug enantiomers may possess different pharmacokinetic characteristics (Drayer 1986; Jamali et al 1989; Tucker & Lennard 1990). As a result, the determination of enantiomers in biological fluids to find the pharmacokinetic parameters of these drugs has become important in the pharmaceutical industry.

A literature survey showed that, so far, no pharmacokinetic studies have been reported on the enantiomers of methocarbamol. This may be due, in part, to the lack of a simple and rapid stereospecific analytical method. Alessi-Severini et al (1992) reported an HPLC technique for the stereospecific determination of methocarbamol in biological fluids using (+)-*S*-naphthylethyl-isocyanate as a chiral derivatizing reagent. The derivatization reaction of the method was accomplished after heating for 12 h at 85°C.

In this study, we report on a new reversed-phase HPLC technique for the resolution of methocarbamol enantiomers after derivatization with (−)-menthylchloroformate (Figure 1).

Materials and Methods

Chemicals

Racemic methocarbamol (Whitehall-Robins, Mississauga, Canada), (+)- *R*-methocarbamol and (−)-*S*-methocarbamol (Lee Labs, Petersburg, VA) were kindly donated by Dr F. Jamali (University of Alberta, Edmonton, Alberta, Canada). The (−)-menthylchloroformate reagent was purchased from Fluka (Buches, Switzerland). All other solvents and chemicals were of either LC or analytical-reagent grade from Merck (Darmstadt, Germany).

Chromatographic conditions

The HPLC system consisted of a 510 HPLC pump, a 480 UV detector and a 476 integrator, all from Waters (Milford, MA). Chromatographic separation of methocarbamol diastereomers was carried out at room temperature on two Resolve C18 columns (3·9 × 150 mm; Waters) connected in series. The mobile phase consisted of phosphate buffer (pH 7·5)–acetonitrile (50 : 50, v/v) pumping at a flow rate of 1 mL min^{-1}. The mobile phase was prepared daily and degassed under vacuum.

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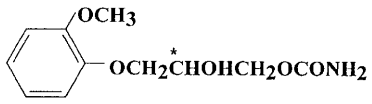
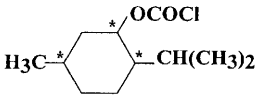


Racemic methocarbamol



Racemic menthylchloroformate

Figure 1. Chemical structure of racemic methocarbamol and menthylchloroformate.

Detection of the derivatives was performed at 274 nm.

Standard solutions

A stock solution of racemic methocarbamol was prepared by dissolving 10 mg methocarbamol in 10 mL water to give a final concentration of 1000 μg mL^{-1} (solution 1). A solution of 100 μg mL^{-1} (solution 2) was prepared by diluting the stock solution with acetonitrile. All solutions were stored at 4°C.

A solution of (−)-menthylchloroformate was made by diluting 1 mL (−)-menthylchloroformate with sufficient toluene to produce 10 mL. The reagent solution was stored at −20°C until just before use. Phosphate buffer (0·05 M) was prepared by dissolving 6·80 g monobasic potassium phosphate in approximately 800 mL distilled water. The pH was adjusted to 7·5 with 1 M sodium hydroxide solution and water was added to produce 1000 mL.

A solution of 1% pyridine was prepared in toluene.

Sample preparation

In a test tube, 100 μL methocarbamol (solution 2) was evaporated to dryness. Pyridine solution (20 μL) and (−)-menthylchloroformate solution in toluene (50 μL) were added. The mixture was vortexed and allowed to react for 60 min. The reaction mixture was evaporated to dryness. The residue was dissolved in 200 μL acetonitrile and 25 μL of the resulting solution was injected onto the HPLC system.

Derivatization yield

Samples of methocarbamol (solution 2; 100 μL) were added to six test tubes and evaporated to dryness. To a set of three samples, 50 μL (−)-menthylchloroformate reagent and 20 μL pyridine were added. To the remaining three samples, 20 μL pyridine and 50 μL toluene were added. The peak areas of methocarbamol in derivatized samples were compared with those of underivatized samples. The experiment was performed on three consecutive days and the mean reaction yield was calculated.

Results and Discussion

Chiral alcohols are usually more difficult to derive with chiral derivatizing reagents than chiral amines, therefore, successful resolutions of optically active alcohols are rather more limited (Zhou et al 1994).

The (−)-menthylchloroformate reagent has been used frequently for the resolution of chiral amines with HPLC techniques (Mehvar 1989 a, b; Prakash et al 1989 a, b; Ahnoff et al 1990). However, only a limited number of its applications on the separation of chiral alcohols or phenols has been reported (Westly & Halpern 1968; Jeyaraj & Porter 1984; Zhou et al 1994). Under anhydrous conditions, menthylchloroformate reacts with alcohols to produce carbonate diastereomers in the presence of pyridine as a catalyst. The carbonate diastereomers can be resolved by reversed-phase HPLC systems which makes menthylchloroformate very favourable for the analysis of chiral alcohols in biological samples.

In this study, precolumn derivatization by menthylchloroformate was used for the separation of methocarbamol enantiomers. Various columns and different combinations of mobile phases were tested to find the optimum condition of chromatography. No satisfactory results were obtained using either the μ Bondapak C18 (3·9 × 300 mm; Waters) or the Novapak C18 cartridge column (3·9 × 150 mm; Waters). Poor resolution was achieved by using the Resolve C18 column. The highest degree of resolution was achieved using two connected columns in series and phosphate buffer (pH 7·5)–acetonitrile (50 : 50, v/v) as the mobile phase, as described above. Using this solvent system two equally sized peaks of (+)-*R* and (−)-*S* diastereomers were obtained at 38·3 and 39·9 min (α = 1·04), respectively (Figure 2). The elution order of the diastereomers was identified by using individual enantiomers. Although complete baseline resolution was not achieved for the derivatives of methocarbamol, the two peaks were integrated easily and accurately. Comparing the chromatograms of the reagent and reagent plus methocarbamol under the same conditions, no interfering peak due to the reagent or its by-products was observed.

Optimal conditions were selected after studying the various parameters affecting the derivatization reaction. Different solvents and catalysts, various

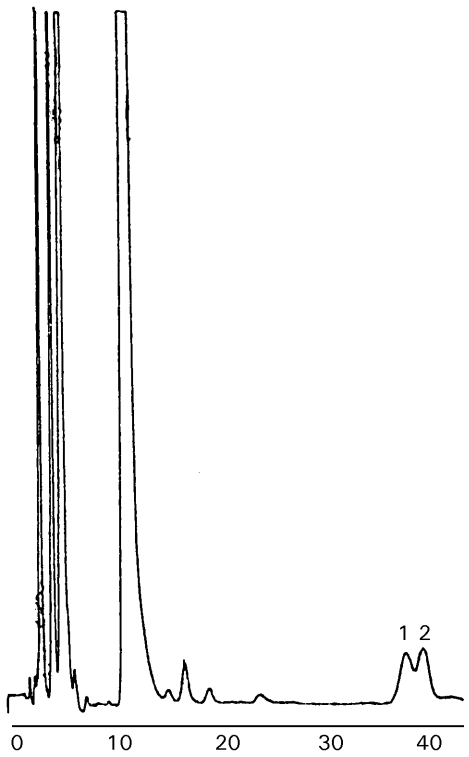


Figure 2. HPLC chromatogram of (−)-menthylchloroformate and methocarbamol after derivatization. Peaks 1 and 2 are (+)-*R*- and (−)-*S*-methocarbamol-(−)-menthylchloroformate, respectively.

amounts of menthylchloroformate, and different times and temperatures were considered.

Among the solvents tested, the most satisfactory result was obtained when toluene was used as the reaction medium. Triethylamine, pyridine and *N*, *N*-dimethylaminopyridine were used as catalysts. The best result was achieved with pyridine, with no interfering peak and better derivatization yield. Therefore, toluene and pyridine were selected as the medium for the reaction. To find the optimum amount of menthylchloroformate, different mole ratios of (−)-menthylchloroformate to methocarbamol (115 : 1, 230 : 1, 345 : 1, 460 : 1, 575 : 1 and 690 : 1) were examined. The ratio of 575 : 1 gave satisfactory results and was chosen for the derivatization reaction.

The reaction was allowed to proceed at different time intervals (5, 30, 60, 90 and 120 min; n = 3). The results showed that maximum peak intensity was observed after 60 min. The same experiment was repeated at 45 and 80°C, and the same result (60 min) was achieved.

The underivatized peak of methocarbamol was detected under the chromatographic conditions used for the separation of diastereomers. The derivatization yield was calculated by comparing the peak areas of methocarbamol for derivatized and underivatized samples. The derivatization yield of methocarbamol under these conditions at room temperature was approximately 57%.

Very few investigations have been carried out on the stereospecific pharmacokinetics of methocarbamol or on the analytical techniques for this valuable drug. This study is probably the first attempt to use (−)-menthylchloroformate to develop a simple method for separation of methocarbamol enantiomers. The derivatization yield of this method does not make it suitable for analytical purposes.

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