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Note

Supercooled liquid crystal gas chromatographic separation of the 2,4- and 2,5-xylyloxy isomers of gemfibrozil and xylenol

JEROME E. HAKY*, BOGUSLAWA LEJA and HORST G. SCHNEIDER
Chemistry Department, Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48106 (U.S.A.)
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Gemfibrozil is the generic name for 2,2-dimethyl-5-(2,5-xylyloxy)-valeric acid, which has been demonstrated to be a safe and effective hypolipemic agent[1]. A potential contaminant in this drug is its 2,4-xylyloxy isomer, which would be the direct result of 2,4-xylenol contamination in the 2,5-xylenol from which the gemfibrozil was synthesized.

Gas–liquid chromatographic (GLC) determination of 2,4-xylyloxy isomer contamination in either gemfibrozil or 2,5-xylenol is difficult, owing to the close similarities in the physical properties of the isomeric compounds. GLC separation of 2,4-xylenol and 2,5-xylenol has been accomplished only through the utilization of highly specialized stationary phases, such as phthalates[2], sugar derivatives[3], and most recently, alkali-metal salts[4,5]. We report here the use of a popular commercially available liquid crystal stationary phase, N,N′-bis-(*p*-methoxybenzylidene)-α,α′-bi-*p*-toluidine (BMBT) for the efficient GLC separations of the easily prepared methyl esters of gemfibrozil and its 2,4-xylyloxy isomer and the benzyl ethers of 2,4-xylenol and 2,5-xylenol, both with sufficient resolution for the quantitative determination of 2,4-xylyloxy isomer contamination in either the drug or its 2,5-xylenol precursor.

EXPERIMENTAL

Materials

The liquid crystal stationary phase, 2.5% BMBT on 100–120 mesh Chromosorb W HP, was obtained from Alltech (Arlington Heights, IL, U.S.A.). The alkylating reagent dimethylformamide (DMF)–dimethylacetal (2 mequiv./ml in pyridine) was obtained from Pierce (Rockford, IL, U.S.A.) and the benzylating reagent benzyl bromide was obtained from Aldrich (Milwaukee, WI, U.S.A.).

Experimental and bulk samples of 2,4-xylenol and 2,5-xylenol were obtained from Aldrich, while samples of gemfibrozil and 2,2-dimethyl-5-(2,4-xylyloxy)valeric acid were synthesized in these laboratories using published procedures[6].

Derivatization reactions

Methyl esters of gemfibrozil and its 2,5-xylyloxy isomer. The sample to be analyzed (2 mg) was placed in a vial containing 250 μl of a solution of DMF–dimethyl-

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acetal in pyridine (2 mequiv./ml) and shaken until dissolved. This solution was used directly for GLC analysis.

Benzyl ethers of 2,4-xylenol and 2,5-xylenol. To 1 ml of methylene chloride containing 4 mg of the sample to be analyzed was added 1 ml of an aqueous solution which was 0.1 *M* in tetrabutylammonium hydrogen sulfate and 0.2 *M* in sodium hydroxide. Benzyl bromide (20 μl) was then added and the mixture was shaken for 20 min. Aliquots of the isolated methylene chloride phases were used directly for GLC analysis.

Apparatus and procedure

GLC analyses were performed on a Perkin-Elmer Model 910 gas chromatograph equipped with a flame ionization detector (FID) and a 6 ft. × 2 mm I.D. glass column packed with the BMBT stationary phase. Chromatograms were recorded and processed on a Varian Model 401 data system. Carrier gas (nitrogen) flow-rate was set at 10 ml/min, while hydrogen and air flow-rates were set at 30 and 300 ml/min, respectively. Solutions (5 μl for the gemfibrozil derivatives and 2 μl for the xylenol derivatives) were injected with a Hamilton 701N 10-μl syringe.

Routinely, samples of the gemfibrozil derivatives were analyzed at a column temperature of 135°C, while samples of the xylenol derivatives were analyzed at 160°C. In all cases, the column temperature was set by first heating the column to 200°C and then cooling to the operating temperature over a 10-min period.

Separation factors shown in Fig. 1 were calculated by the ratio of corrected retention times, using butane as an unretained substance for the determination of dead volumes.

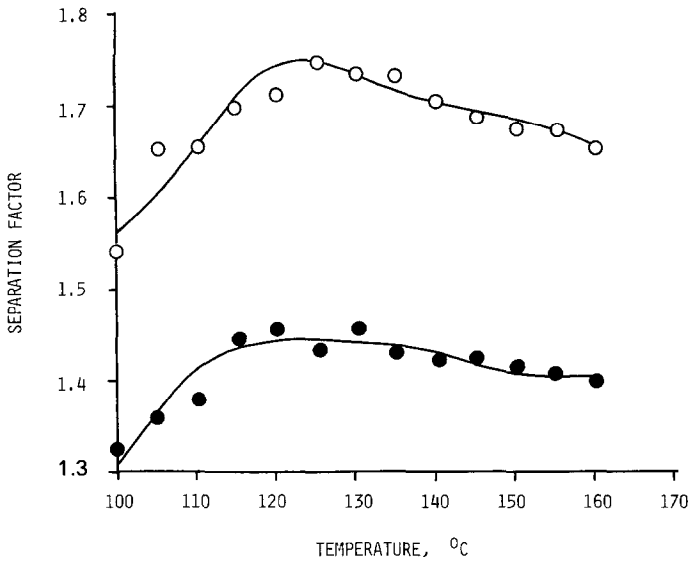

Fig. 1. Behavior of the separation factor with temperature on the BMBT column. ● = Gemfibrozil and 2,2-dimethyl-5-(2,4 xylyloxy)valeric acid methyl esters; ○ = 2,4-xylenol and 2,5-xylenol benzyl ethers.

RESULTS AND DISCUSSION

The liquid crystal stationary phase BMBT has been widely used for the GLC separation of positional isomers of a number of high-boiling compounds, including steroids[7], polychlorinated biphenyls[8], and 3–5-ring polycyclic aromatic hydrocarbons[9]. More recently, the ability of this stationary phase to remain in a supercooled liquid crystalline state below its normal solid–nematic transition temperature (181°C) has enabled it to be used at lower column temperatures for the analysis of more volatile 2-ring aromatic compounds, such as the isomers of dimethylnaphthalene[10,11]. It is this supercooling property, coupled with the proper choice of derivatizing reactions, which has now enabled BMBT to be used for the GLC separation and analysis of the single-ring aromatic compounds under study here.

Fig. 1 shows the behavior of the separation factors, α, between the 2,4- and 2,5-xylyloxy isomers of both the gemfibrozil methyl esters and the xylenol benzyl ethers, as the column is cooled in 5°C increments. For both sets of isomers, maximum separation occurs near the temperature corresponding to the supercooled nematic → solid phase transition for BMBT (*ca.* 120–125°C), which is consistent with previous

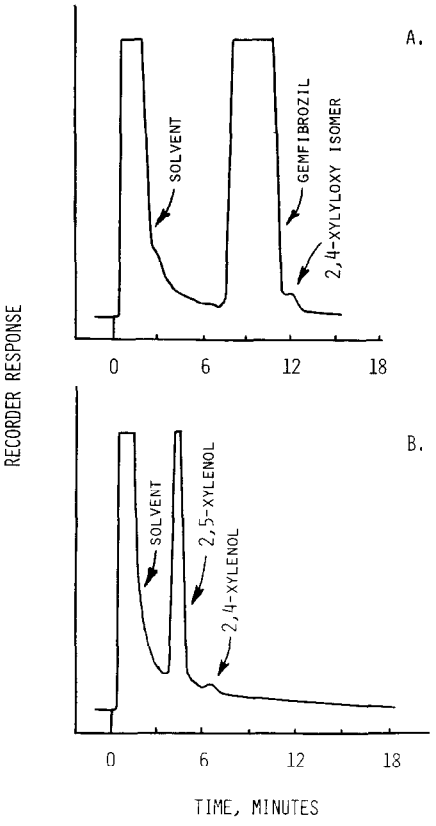

Fig. 2. Representative chromatograms from the GLC analysis of bulk lots. A, Gemfibrozil containing 0.3% 2,4-xylyloxy isomer contamination, column temperature = 135°C. B, 2,5-xylenol containing 0.7% 2,4-xylenol contamination, column temperature = 160°C.

studies of separations of dimethylnaphthalenes on the BMBT stationary phase[10,11]. At column temperatures below this phase transition, separation becomes poor and chromatographic peaks badly broaden, due to the loss of liquid crystalline order and selectivity in the stationary phase.

Separation of isomers on liquid crystal stationary phases has been ascribed to a geometric mechanism based on the retention of compounds in the order of their molecular length-to-breadth ratios[12]. The elution of the longer 2,4-xylyloxy isomers of gemfibrozil and xylenol subsequent to their corresponding 2,5-xylyloxy isomers on the BMBT column in this study is consistent with this mechanism. Moreover, the greater separation observed for the xylenol isomers than for the gemfibrozil isomers (Fig. 1) also follows this mechanism, since the flat, relatively rigid benzyl derivatives of the xylenol isomers would be expected to have greater differences in their average molecular length-to-breadth ratios than the flexible, branched isomers of the methyl ester of gemfibrozil. In any event, separation of either set of isomers is sufficient for the quantitative determination of 2,4-xylyloxy isomer present, although a lower column oven temperature is required to accomplish this determination in the analysis of gemfibrozil samples.

Representative chromatograms from the GLC analyses of bulk lots of both gemfibrozil and 2,5-xylenol on the BMBT column are shown in Fig. 2. Both show the presence of trace amounts (less than 1%) of 2,4-xylyloxy isomer contamination. Since FID detector response is virtually identical for isomeric compounds of this type[13], quantitation of the amount of the 2,4-xylyloxy isomer present is readily accomplished through area or height-percent calculations. The limit of detection for 2,4-xylyloxy isomer content by this procedure is at least 0.1% for either gemfibrozil or 2,5-xylenol.

CONCLUSIONS

In this study, utilization of the supercooled nematic thermal region of the stationary phase BMBT has allowed its use in the GLC determination of positional isomer contamination in bulk lots of a single-ring aromatic drug and its synthesis precursor. The utility of such a liquid crystal stationary phase in helping to define the isomeric purity of a bulk drug demonstrates a potential application for such stationary phases which has not been fully exploited. Investigations in these laboratories on similar applications are continuing.

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