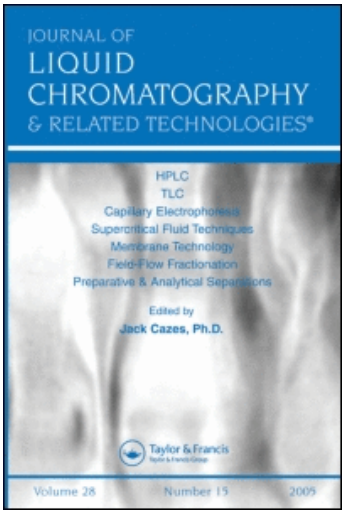

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Chromatographic Behavior of the Anthelmintic Fenbendazole and Its Major Metabolite Oxfendazole in Various Ion-Pair Liquid Chromatographic Systems

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CHROMATOGRAPHIC BEHAVIOR OF THE ANTHELMINTIC FENBENDAZOLE AND ITS MAJOR METABOLITE OXFENDAZOLE IN VARIOUS ION-PAIR LIQUID CHROMATOGRAPHIC SYSTEMS

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ABSTRACT

The chromatographic behavior of fenbendazole (FBZ) and oxfendazole (OFZ) in various reversed-phase liquid chromatographic (LC) systems has been investigated. The addition of negative and/or positive charged ion-pair reagents in the mobile phase has been examined, whereas the influence of mobile phase pH, mobile phase composition, and column temperature on retention and peak height has been evaluated. The observed behavior of the analytes during the various chromatographic processes has been discussed.

INTRODUCTION

Various LC systems for analyzing residues of the anthelmintic FBZ and its major metabolite OFZ (Fig. 1) in food of animal origin have been described. Most are ion-suppression systems [1-5], whereas a few are ionization enhancement systems [6-8]. Owing to the significant polarity difference of the compounds, excessive retention and peak broadness of the late eluted FBZ is noted in ion-suppression systems. In ionization enhancement systems, the retention time of FBZ is considerably shortened due to its protonation but severe peak tailing occurs as a result of the residual free silanol action on the stationary phase. Two approaches have been developed to overcome these problems. Some workers used, as an alternative to gradient elution, a weak elution strength ion-suppression mobile phase to isocratically analyze OFZ and, in succession, a much higher elution strength mobile phase to quickly elute FBZ [2-4]. Others suggested addition of pentanesulfonate and/or triethylamine pairing ions into an ionization enhancement [9] or ion-suppression [10] mobile phase, respectively, in order to better control the selectivity and elute the analytes in a single LC run without tailing. Both approaches are useful in LC analysis of these benzimidazoles but the variables affecting the chromatographic behavior of these compounds in such LC systems remain to be studied.

This paper reports on the chromatographic behavior of FBZ and OFZ in various LC systems. Using mobile phases containing or not negative and/or positive charged ion-pair reagents, the influence

A B

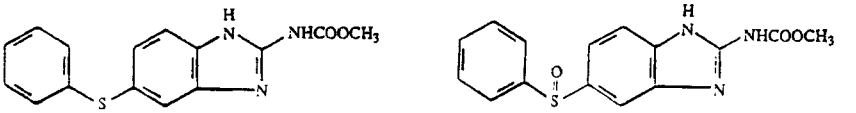

Figure 1. Structure of FBZ (A) and its major metabolite OFZ (B).

of pH, organic modifier content and column temperature on retention and peak height of the analytes is investigated.

EXPERIMENTAL

Instrumentation

LC was carried out on a Gilson system consisting of a Model 805 manometric module, a Model 305 piston pump, a Model HM/HPLC dual-beam variable-wavelength spectrophotometer set at 293 nm, a Model 831 column oven, and a model N1 variable-span recorder (Villiers-le-Bel, France). Injections were made on a Hichrom, 250x4.6 mm, stainless-steel column packed with Nucleosil 120 C_{18}, 5-µm, through a Rheodyne 7125 sample injector equipped with a 100-µl loop.

Chemicals

Octanesulfonate (OS) sodium salt, tetrabutylammonium (TBA) hydrogen sulfate, and LC grade acetonitrile and water were purchased from Merck-Schuchard (Germany). Standard OFZ was

donated from Hoechst (Germany) whereas standard FBZ was obtained from Riedel-de Haen (Germany).

Stock solutions of the individual benzimidazoles (ca. 100 μg/ml) were prepared by dissolving each standard in 10 ml dimethylsulfoxide and diluting to 100-ml volume with acetonitrile. Mixed working solutions were prepared by diluting appropriate aliquots of the stock solutions of FBZ and OFZ in the mobile phase used each time.

Chromatographic Conditions

The mobile phases used were all mixtures of acetonitrile and 0.01 M phosphate buffer (25:75, 35:65 or 40:60, v/v) in the pH range 2.2-6.5, containing or not OS and/or TBA as ion-pair reagents. Addition of the ion-pair reagents was carried out in the phosphate buffer so as their final concentration in the mobile phase to be 5 mM for TBA or 10 mM for OS addition, and 5 mM TBA plus 5 mM or 10 mM OS co-addition. Following addition of the tested pairing ion, the pH of the phosphate buffer was adjusted using 1 M phosphoric acid or sodium hydroxide solution. The mobile phase was degassed using helium and delivered at a rate of 1 ml /min.

The LC column was thoroughly equilibrated with mobile phase each time before use. Reproducible capacity factors (k′) could be obtained after passage through the column of at least 70 ml of mobile phase. When the mobile phase contained ion-pair reagents, passage of 150-ml volume was indispensable for column equilibration. On changing the mobile phase, successive column washing with at least 100 ml portions of water and acetonitrile was

found to be effective for removing the adsorbed pairing ions. Recordings were made at a chart speed of 5 mm/min and a recorder setting of 0.020 a.u.f.s.

RESULTS AND DISCUSSION

Influence of mobile phase pH and ion-pair reagent on retention and peak height of FBZ and OFZ

The effect of the mobile phase pH and ion-pair reagents on retention and peak height of FBZ and OFZ was investigated using the Nucleosil 120 C_{18}, 5 μm, stationary phase equilibrated at ambient temperature (~20 °C). The mobile phases used were all mixtures of acetonitrile and 0.01 M phosphate buffer (40:60, v/v) in pH range 2.2-6.5, containing or not OS and/or TBA reagents. Owing to the wide pH range of the mobile phases, a detection wavelength of 293 nm was selected to compensate for absorption differences between protonated and unprotonated OFZ; the absorption maximum of this compound undergoes a remarkable red shift (291.2 nm to 295.5 nm) at pH values higher than 3 (Fig. 2). Such a shift was not observed in the case of FBZ.

Effect of mobile phase pH and ion-pair reagent on retention

The effect of the mobile phase pH and pairing ion (type and concentration) on the capacity factors of FBZ and OFZ are shown in Figures 3 and 4, respectively. When the pH of the mobile phase ranged between 3.7 and 6.5, the capacity factors of both

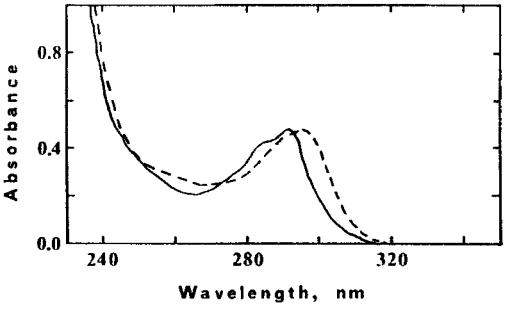

Figure 2. UV absorption spectra of standard OFZ (7.7 μg/ml) in 40% acetonitrile in 0.01 M phosphate buffer adjusted at pH 2.2 (full line) or pH 6.5 (broken line).

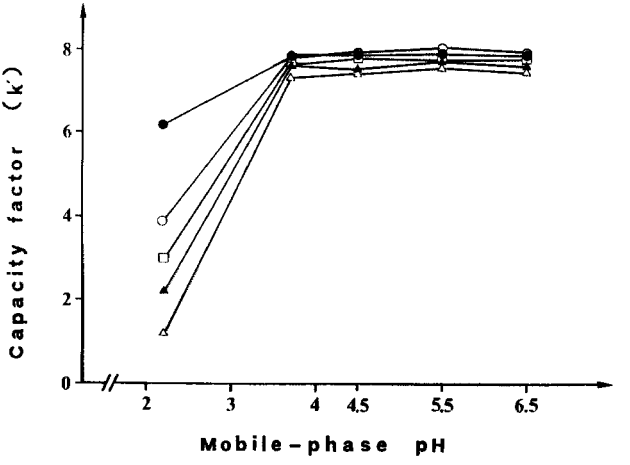

Figure 3. Influence of mobile-phase pH and pairing ion on the capacity factors of FBZ. Chromatographic conditions: stationary phase, Nucleosil 120 C_{18}, 5 μm; mobile phase, acetonitrile/0.01 M phosphate buffer (40:60, v/v) in pH range 2.2-6.5 (○) containing 10 mM OS (●), 5 mM TBA (△), 5 mM TBA + 5 mM OS (▲), and 5 mM TBA + 10 mM OS (□); column temperature, 20 °C; flow rate, 1 ml/min; wavelength, 293 nm.

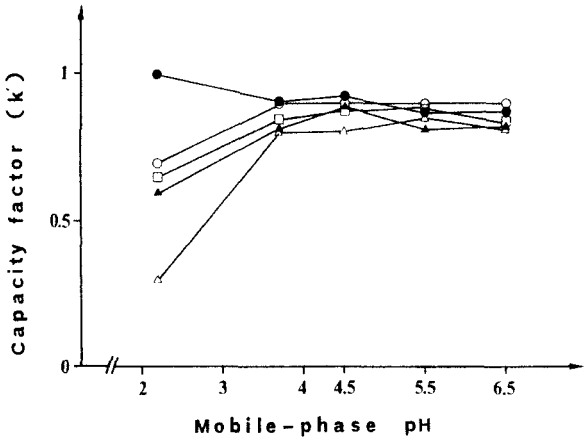

Figure 4. Influence of mobile-phase pH and pairing ion on the capacity factors of OFZ. Chromatographic conditions and curve symbols as shown in Figure 3.

benzimidazoles were not affected by pH value. Addition of negatively charged OS and/or positively charged TBA ions had also no considerable effect on retention due, obviously, to suppression of the ionization of FBZ and OFZ molecules in this pH range.

Decreasing the pH of the mobile phase to 2.2, protonation of FBZ and OFZ molecules occurs. As a result of it, the solubility of both analytes in the mobile phase was increased, thereby sharply reducing the column retention of the late eluted FBZ and slightly that of the early eluted OFZ. The retention decrease for FBZ varied with both the presence and the type of the ion-pair reagent being lower in the case of OS anions, more pronounced in absence of pairing ions, higher in presence of both OS anions and TBA cations, and arrived its maximum when only TBA cations were present in

the pH 2.2 mobile phase. OFZ exhibited a similar retention behavior in all cases except that of OS addition where a slight retention increase was noted instead.

The maximum retention noted in case of OS addition indicated ion-pairing of the OS anions and positively charged benzimidazoles to more hydrophobic forms. On the other hand, the minimum retention observed in case of TBA addition could be partly at least due to efficient masking of the negatively charged residual silanols by the TBA cations [11-12]. Electrostatic repulsion of the protonated benzimidazoles by the TBA cations adsorbed on to the octadecylsilica surface might also contribute to this effect [13-14].

The retention enhancement noted when the mobile phase in addition to TBA cations contained equal or higher concentration of OS anions was difficult to explain. The retention mechanism in such chromatographic systems has not yet been elucidated. The alkanesulfonate may interact with both the anti-tailing quaternary ammonium ions and solute ions; further, the two opposite charge surfactants may be co-adsorbed on to the column material [15-17]. Figures 3 and 4 indicate that the affinity of TBA cations to residual silanols is more pronounced than that to OS anions, as the anti-tailing effect of TBA is not reduced by the OS presence. These observations lend support to previous findings suggested by other workers [18]. Figures 3 and 4 also suggest that negatively charged counter ions capable to form ion pairs with solute cations are present even in case the concentration of the alkanesulfonate is not higher than that of the quaternary ammonium compound.

Therefore, the effect of variations in the concentration of the OS anions can be expected to be as in reversed phase ion pair chromatography; further enhancement of retention occurs when the concentration of OS anions is twice than that of TBA cations.

Effect of mobile phase pH and ion-pair reagent on peak height

The effect of the mobile phase pH and pairing ion (type and concentration) on peak height of FBZ and OFZ are shown in Figures 5 and 6, respectively. In absence of ion-pair reagents, broad and low height peaks were consistently taken for the late eluted FBZ at any mobile phase pH. At pH 2.2, where FBZ is in its protonated form., the distortion was more pronounced as severe peak tailing also appeared due, obviously, to strong silanophilic interactions with the stationary phase (Fig. 7).

Addition of negatively charged OS and/or positively charged TBA ions had a spectacular effect on both the height (Fig. 5) and the shape (Fig. 8) of FBZ peak. Even in cases of excessive column retention, peak shape was greatly improved, peak heights were markedly increased, and peak distortion was totally eliminated.

Influence of column temperature and organic modifier content on retention and peak height of FBZ and OFZ

The influence of column temperature and organic modifier content on retention and peak height of FBZ and OFZ was investigated using different mixtures of acetonitrile and 0.01 M phosphate buffer pH 2.2, containing 5 mM TBA and 5 mM OS. The

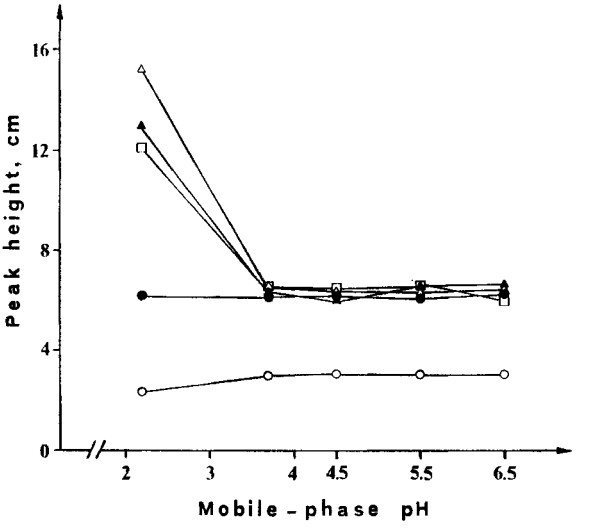

Figure 5. Influence of mobile-phase pH and pairing ion on peak height of FBZ. Chromatographic conditions and curve symbols as shown in Figure 3.

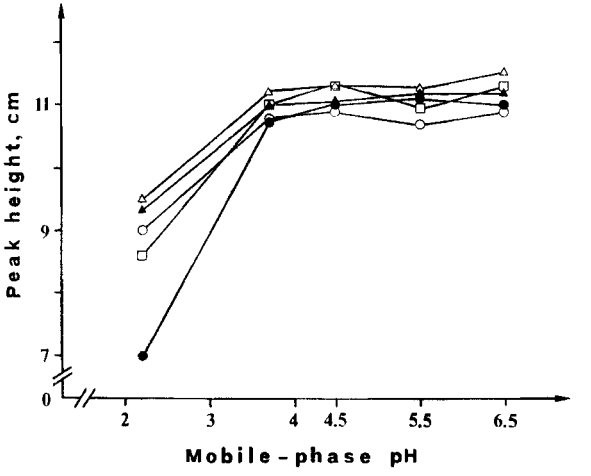

Figure 6. Influence of mobile-phase pH and pairing ion on peak height of OFZ. Chromatographic conditions and curve symbols as shown in Figure 3.

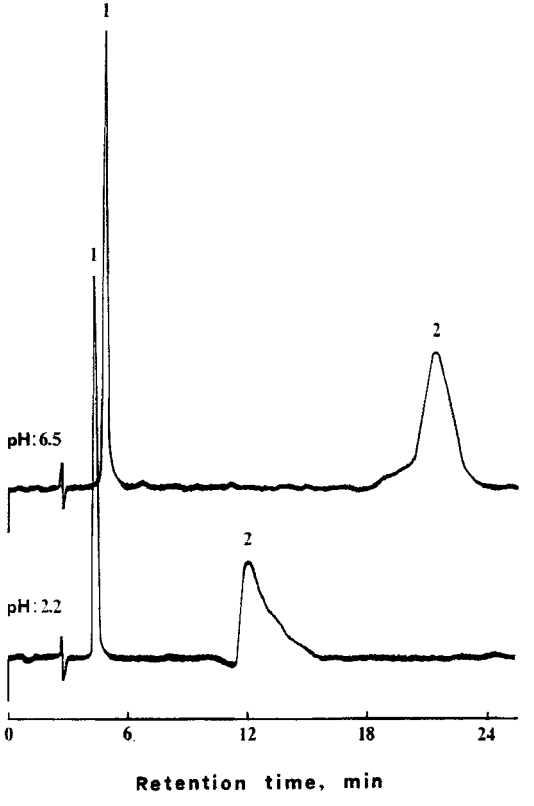

Figure 7. Typical chromatograms of standard solutions containing 0.6 μg/ml OFZ (1) and 1.1 μg/ml FBZ (2). Chromatographic conditions: stationary phase, Nucleosil 120 C_{18}, 5 μm; mobile phase, 40% acetonitrile in 0.01 M phosphate buffer adjusted at pH 2.2 or 6.5; column temperature, 20 °C; flow rate, 1 ml/min; wavelength, 293 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 5 mm/min; injection volume, 100 μl.

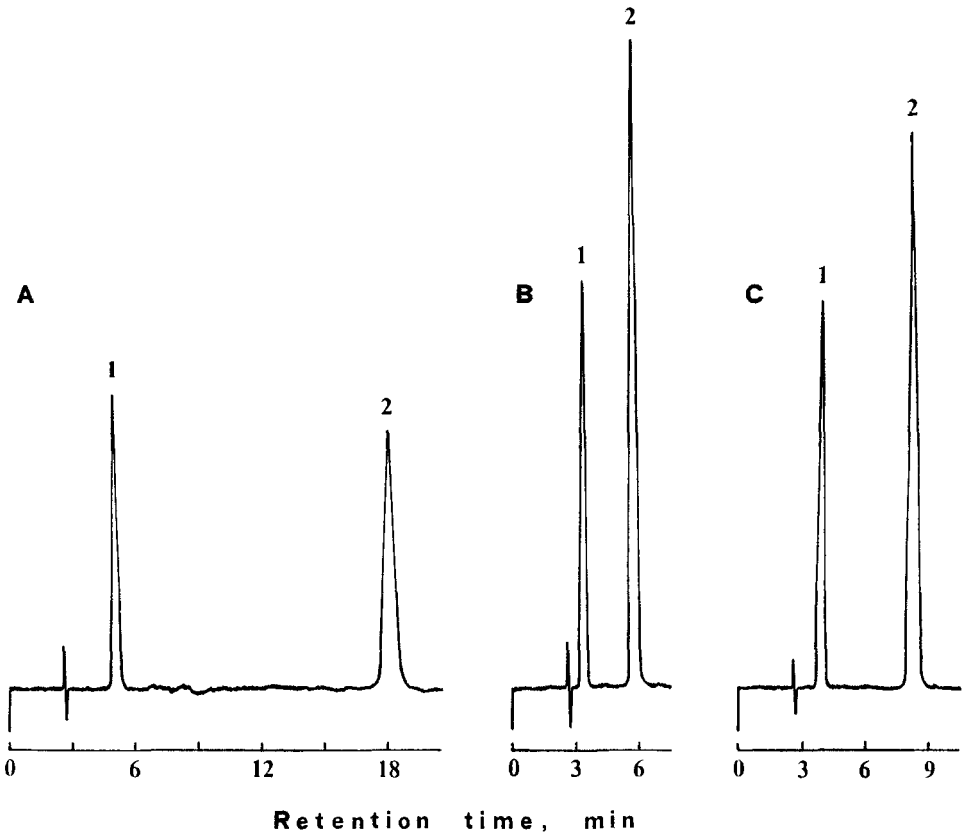

Figure 8. Typical chromatograms of standard solutions containing 0.6 μg/ml OFZ (1) and 1.1 μg/ml FBZ (2). Mobile phase, acetonitrile/0.01 M phosphate buffer (40:60, v/v) pH 2.2, containing 10 mM OS (A), 5 mM TBA (B), and 5 mM TBA + 5 mM OS (C). Other chromatographic conditions as shown in Figure 7.

changes in k′ values as a function of column temperature and organic modifier content for mobile phases containing 25%, 35%, and 40% acetonitrile are shown in Figure 9.

Increasing the concentration of acetonitrile in the mobile phase, decreased capacity factors were taken for both

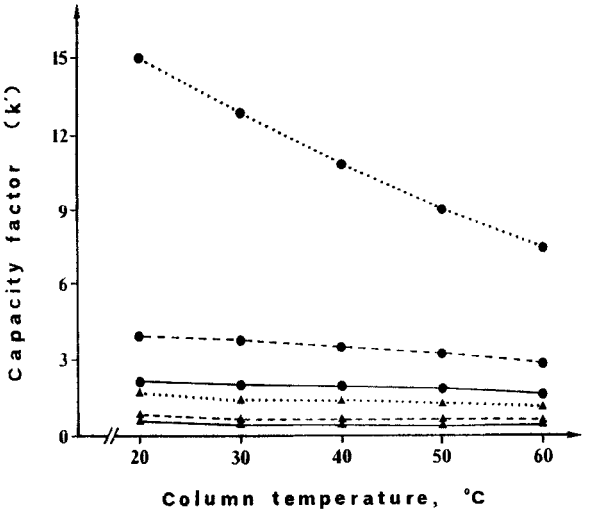

Figure 9. Influence of column temperature and organic modifier content on capacity factors of FBZ (●) and OFZ (▲). Mobile phase, 25% (dotted lines), 35% (broken lines), and 40% (full lines) acetonitrile in 0.01 M phosphate buffer pH 2.2 containing 5 mM TBA + 5 mM OS; column temperature ranged from 20 °C to 60 °C. Other chromatographic conditions as shown in Figure 3.

benzimidazoles at any column temperature. This reduction of retention, being more pronounced at 20 °C, had a significant effect on peaks of both analytes. Peak shape was considerably improved and peak heights were markedly increased when the mobile phase composition changed from 25% to 40% acetonitrile.

Increasing column temperature up to 60 °C for any mobile phase composition, a progressive but reasonable reduction of OFZ retention was noted. FBZ exhibited a similar behavior in case the mobile phase contained 35% or 40% acetonitrile whereas at lower (25%) concentration its retention was considerably affected by

column temperature; changing temperature from 20 °C to 60 °C, the retention decreases up to 50%. Increasing column temperature exerts also a beneficial effect on peak heights. This effect was more pronounced when the mobile phase contained 25% acetonitrile and moderate at 35% acetonitrile, a finding which suggests that control of temperature may be of help in specific ion-pair separations. It is of interest, however, to note that this effect was almost negligible when the mobile phase contained 40% acetonitrile.

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