ION-PAIRING CHROMATOGRAPHY OF LEUKOTRIENES LTB_4, LTC_4 AND LTD_4

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The leukotrienes are a group of structurally related compounds produced during the catabolism of arachidonic acid. They can be detected by U.V. absorption subsequent to hplc separation, Osbourne et al (1983). It has been suggested, Metz et al (1982), that the use of ion pairing might be advantageous in the separation of these compounds. To date in the literature all hplc separations reported have been based on pH control using reverse phase systems.
Figure 1 shows separations using a 100 x 2.1 mm column packed with ODS Hypersil (5µm) stationary phase as a function of concentration of tetrabutylammonium (TBA) pairing ion at pH 7. All compounds are retained at 40 mM TBA and good selectivity is shown between all pairs of compounds. Figure 2 shows the separations obtained when an anionic pairing ion lauryl sulphate (SLS) is used at pH 2.2. Only the potentially basic LTC_4 and LTD_4 are retained by this pairing ion. The retention of LTB_4 which is neutral at this pH is reduced by addition of SLS. These results are consistent with modern ideas of ion pairing, Taylor et al 1984.

Figure 1. CH_3CN:0.01M Buffer 35:65 pH 7.0

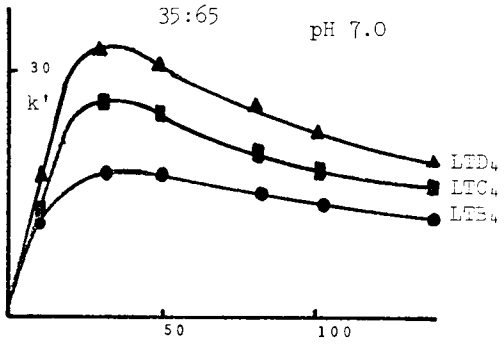
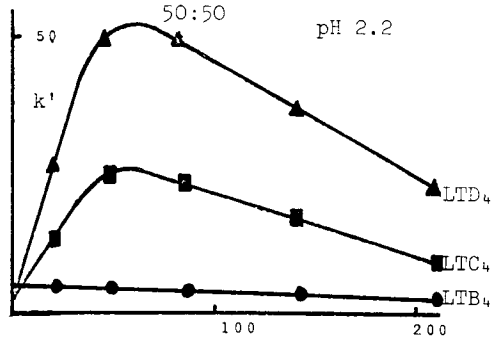


Figure 2. CH_3CN:0.01M Buffer 50:50 pH 2.2



Since no quantitative stability data could be located in the literature on these compounds, first order rate constants were determined, using 0^0C as a reference temperature, in water and also in the buffers used in the chromatographic solvents. Leukotriene peak heights were measured as a function of time and the decomposition was treated as following first order kinetics. The results are shown in Table 1. The large errors associated with certain of these values are a consequence of the limited extent of reaction followed. All compounds are more stable in either buffer solution than in water and, in the time scale required for chromatographic separation, no appreciable decomposition should occur at either of the pH values used at the temperature used for chromatography.

First Order Rate Constants k x 10^5 (RSD%)

	Water	pH 7.0	pH 2.2
LTB	103 (11.1)	3.71 (2.5)	2.27 (92)
LTC	633 (5.4)	6.45 (8.5)	6.25 (40)
LTD	834 (7.2)	17.9 (7.9)	4.64 (39)

Table 1
Showing First Order Rate Constants for the Leukotrienes in different solvents at 0^0C.

Other pairing ions used viz cetyltrimethylammonium and tetraethylammonium did not improve selectivity with adequate retention.

Metz, S.A. et al (1982) J. Chromatogr. 233: 193-201.
Osbourne, D.J. et al (1983) Prostaglandins. 26: 817-832.
Taylor, R.B. et al (1984) J. Chromatogr. 316: 279-289.