SHORT NOTE

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Preparative RP-HPLC of ecdysteroids

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Ecdysteroids, RP-HPLC, water-n-alcohols insoeluent systems

HPLC is one of the most powerful technique for analysis and isolation of ecdysteroids (ES). To the purposive search of preparative chromatography conditions we have tried to restrict the variety of partition forces in whole system and have investigated the simplest binary eluents *n*-alcohol/water in appliance to separation of isomeric ES: 20HE and I. Fortunately, ES demonstrate two major types of interactions both in mobile phase and on the surface of a sorbent. It is due to the presence in the molecules hydrophobic hydrocarbon bone and OH-groups with ability to form *H*-bonds. Two substrata distinguish only in OH-position whether at C25 or at C26. All systems were checked in analytical scale and then translated into preparative chromatography (Fig. 1).

It had appeared that binary compositions with different concentrations of alcohols demonstrate very similar retention times, selectivity and R_s towards our sample. Alcohols content for iso-eluent systems were: a) 30–40% CH_3OH, b) 10–20% C_2H_5OH, c) 2–5% C_4H_9OH and d) 0.2–0.6% $C_6H_{13}OH$ (all by volume). It is not surprising if one keeps in mind the following reasons: molecules of alcohols differ each other by length of hydrocarbon radicals but nature remains of solvent-solute and solute-surface interactions; it is known, the bigger R in ROH, the higher energy of alcohol adsorption on RP-surface; hence, to alter sorbent surface in equal and curtain extent it needs different quantity of each alcohol; ES and alcohols are supposed to compete for the same sites on the surface because of the similar chemical nature of the molecules groups. In our conditions all molecular participants of the chromatography process demonstrate really analogous chemical features. The confirming examples are following.

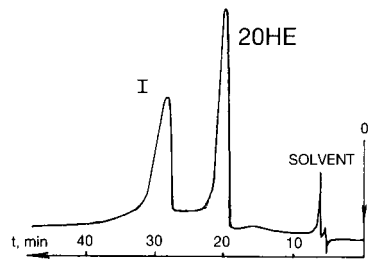

Fig. 1. Preparative separation. Eluent buthanol/water 4 : 96 v/v, column Diasorb-C16/T, 250 × 25 mm ID, 10 mkm particles size, refractometric detecting.

There are experimental data, that substitution of some part of one alcohol by equivalent part of any other (for instance 1/2 of methanol in eluent *a* by 1/2 of buthanol from eluent *c*) forms tertiary mixture with no change in the chromatographic feature of initial isoeluents. Moreover, mixture demonstrates intermediate properties if starting binary solvents are distinguished. Semi-log representation of M, mol%, of alcohols in eluents with similar chromatographic features vs. hydrophobic surface S, $Å^2$, of corresponding ROH molecules is straight line with extra fine accuracy (Fig. 2). Such a result is supposed to originate from the evident connection between energy of alcohols adsorption and properties of corresponding eluents in RP chromatography of ES.

So, modelling chromatographic interactions had allowed us to find a set of the simple and convenient eluents in both analytical and preparative modes, acting according to one type partition mechanism and consequently with predicable chromatographic properties.

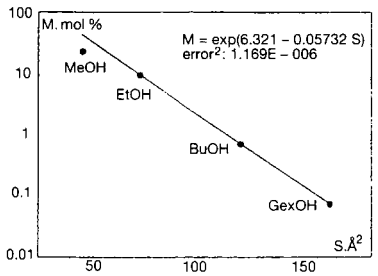

Fig. 2. Connection of alcohols content M, mol%, with non-polar surface of alcohol molecules S, $Å^2$, for iso-eluents.