Journal of Chromatography A, 1440 (2016) 229–239

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Influence of carboxylic ion-pairing reagents on retention of peptides in thin-layer chromatography systems with C18 silica-based adsorbents

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ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 20 February 2016
Accepted 22 February 2016
Available online 24 February 2016

Keywords:
Thin-layer chromatography
Peptide separation
Reversed-phase chromatography
Ion-pairing reagents
Peptide retention

ABSTRACT

One of the main problems related to chromatography of peptides concerns adverse interactions of their strong basic groups with free silanol groups of the silica based stationary phase. Influence of type and concentration of ion-pairing regents on peptide retention in reversed-phase high-performance liquid chromatography (RP-HPLC) systems has been discussed before. Here we present influence of these mobile phase additives on retention of some peptide standards in high-performance thin-layer chromatography (HPTLC) systems with C18 silica-based adsorbents. We prove, that due to different characteristic of adsorbents used in both techniques (RP HPLC and HPTLC), influence of ion-pairing reagents on retention of basic and/or amphoteric compounds also may be quite different. C18 silica-based HPTLC adsorbents provide more complex mechanism of retention and should be rather considered as mixed-mode adsorbents.

1. Introduction

Planar chromatography technique is a well established and popular tool used for separation of many various compounds, including peptides. In recent years it has acquired new, additional potential in the field of analysis of these compounds—due to combination with mass spectrometry, using various methods of sample ionization: matrix-assisted laser desorption/ionization (MALDI) [1–5], desorption electrospray ionization (DESI) [6–8] and electrospray ionization (ESI) [9]. Literature concerning peptide separation shows, that planar chromatography techniques are usually used to analyze relatively simple peptide samples [1–3,8,10–26], but there are also examples of separation of more complex mixtures, such as protein digests [4–7,9,22,27–30]. However, one of main problems related to chromatography of peptides using silica-based adsorbents is concerned with adverse interactions of basic groups of peptide with free silanol groups of the stationary phase. This refers to planar [26] as well as to column reversed-phase systems [31–37]. Therefore to obtain good separation efficiency, suppression of interactions mentioned by use of proper ion-pairing reagents is required.

Despite the fact, that many papers (examples mentioned above) present chromatography of peptides in planar systems, they describe analysis of specific samples and separation conditions rather than influence of particular variables on retention of solutes and separation efficiency of system used. However, proper understanding of influence of ion-pairing reagents on peptide separation in planar chromatography system seems to be crucial for optimization of separation conditions. This is especially curious considering our preliminary results [26]. These suggest, that mechanism of retention with use of commercially available C18 modified silica-based chromatographic plates, may be different, than the one with use of silica-based adsorbents designed for reversed-phase high-performance liquid chromatography (RP-HPLC) of peptides. Reversed-phase columns for peptide separation are usually designed to prevent adverse interactions between basic groups and free silanols of the adsorbent [35–41]. Therefore retention bases almost exclusively on 'hydrophobic interactions'. Results obtained for column reversed-phase systems show that increase of concentration of ion-pairing reagent in the mobile phase results

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http://dx.doi.org/10.1016/j.chroma.2016.02.065
0021-9673/

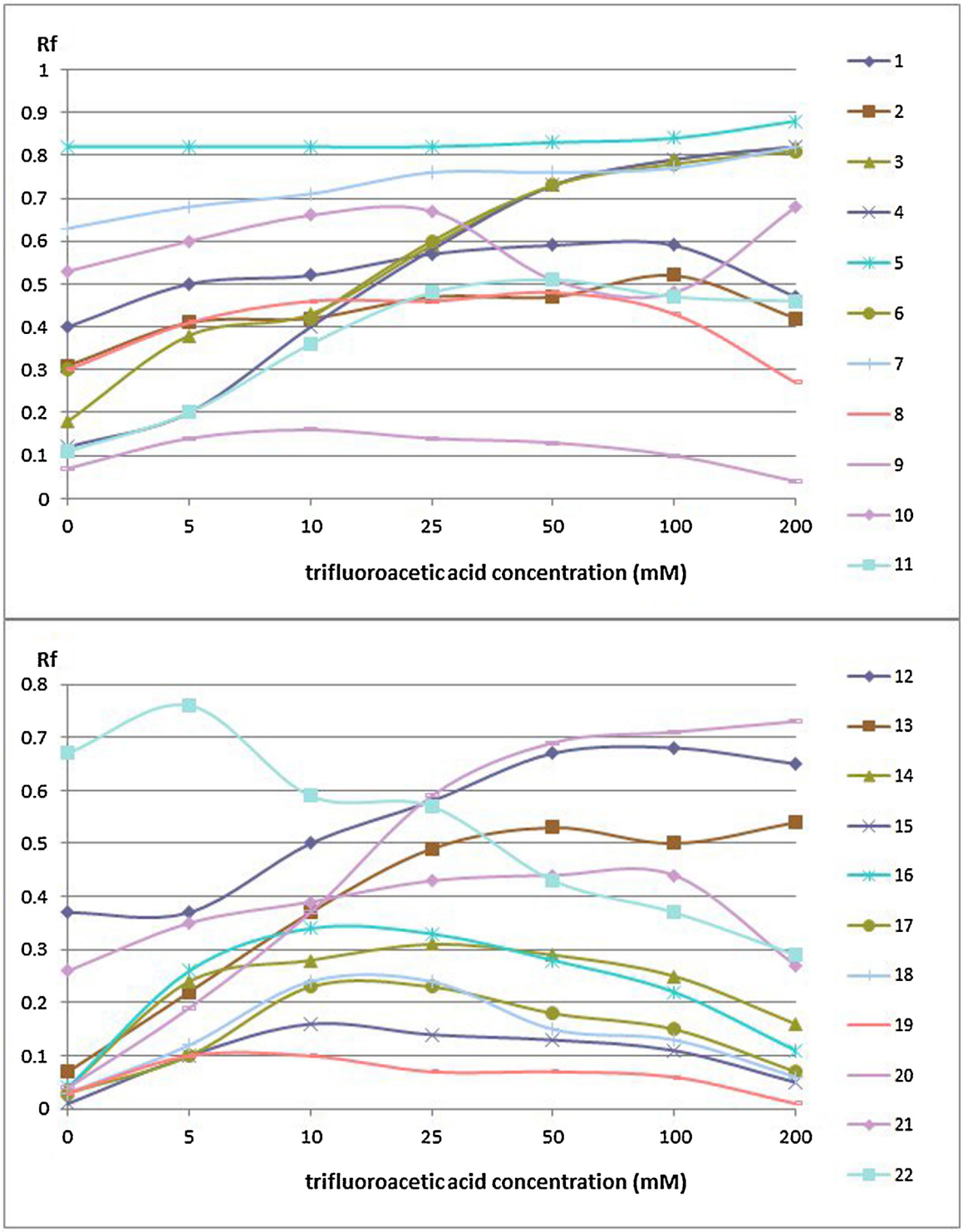

Fig. 1. Relationship: peptide R_f vs. TFA concentration in the mobile phase. Experiments performed using HPTLC RP-18 W chromatographic plates (Merck) and mixture of water/methanol (4/1 v/v) with addition of acid (final concentration indicated on the graph). Numbers of peptide standards indicated on the right side of the figure. Chromatograms development distance was 7 cm.

in increase of peptide retention [31–33]. This is due to blocking of basic (highly polar) groups of these compounds by formation of ion-pair complexes of lower polarity than parent compound. It leads to enhancement of 'hydrophobic interactions' between non-polar adsorbent and solute ion-pair complexes. Our previous results have suggested that RP-18 silica-based adsorbents designed for HPTLC provide complex mechanism of retention, which exhibit mixed-polar and nonpolar character [26,30]. Therefore in HPTLC systems with such adsorbents, decrease of peptide retention with increase of ion-pairing reagent concentration can be observed [26]. So, under certain conditions an effect of ion-pairing reagent on peptide retention in HPTLC systems can be different than that in typical RP-HPLC ones.