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Separation of selected plant origin substances with reversed-phase thin-layer chromatography at controlled mobile phase velocity and stepwise pH gradient

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Abstract

Nowadays, phytotherapy represents one of the most common solutions in the treatment of minor symptoms and major diseases (e.g. Alzheimer's Disease) [1]. In the days of dynamic research for the new plant origin biologically active substances, high-performance thin-layer chromatography, HPTLC, allows rapid and parallel separation of numerous samples. Planar chromatography is readily used for screening analysis of plant extracts, herbal medicinal products, and new substances obtained by biotransformation. Some of those new substances possess acid-base properties (e.g alkaloids). In aqueous solutions, they can dissociate and present diversified interactions of ionized and non-ionized forms of the compound with the mobile phase and stationary phase. A very important tool affecting selectivity and shape of the peaks/spots of such substances is pH. Buffered eluents were commonly used in the separation of substances with ionogenic groups using high-performance column chromatography, HPLC, and planar chromatography, TLC, [2][3] but there are no examples of an eluent pH gradient in reversed-phase high-performance thin-layer chromatography, RP-HPTLC.

Our research group proposes the implementation of the prototype device based on application of moving pipette driven by a 3D printer mechanism to chromatogram development. The device enables developing the pH gradient thin-layer chromatograms in a reversed-phase system with a controlled solvent velocity without excessive flux of the eluent on the surface of the adsorbent layer [4]. In the study, stepwise pH gradient elution has been used for the separation of selected substances showing weak base properties. The use of the pH gradient of the mobile phase has improved the selectivity of separation and shape of the spots/zone of the selected substances.

HPTLC RP-18W chromatographic glass plates (Merck) were used as a stationary phase. The mobile phase was composed of methanol and an aqueous buffer of appropriate pH in the range from 2.5 to 10.5. Obtained pH gradient chromatograms will be shown. The advantages and disadvantages of the proposed solution will be discussed.

Biography:

Adrian Szczyrba, student of the sixth year of pharmacy, member, and leader of Student Research Group. He is interested in separation techniques, advances in chromatography, and application of chromatography techniques in pharmaceutical sciences.

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