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# Ruta graveolens Fingerprints Obtained with Reversed-Phase Gradient Thin-Layer Chromatography with Controlled Solvent Velocity

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**Abstract :** Since prehistory, plants were constituted as an essential source of biologically active substances in folk medicine. One of the examples of medicinal plants is Ruta graveolens L. For a long time, Ruta g. herb has been famous for its spasmolytic, diuretic, or anti-inflammatory therapeutic effects. The wide spectrum of secondary metabolites produced by Ruta g. includes flavonoids (eg. rutin, quercetin), coumarins (eg. bergapten, umbelliferone) phenolic acids (eg. rosmarinic acid, chlorogenic acid), and limonoids. Unfortunately, the presence of produced substances is highly dependent on environmental factors like temperature, humidity, or soil acidity; therefore standardization is necessary. There were many attempts of characterization of various phytochemical groups (eg. coumarins) of Ruta graveolens using the normal – phase thin-layer chromatography (TLC). However, due to the so-called general elution problem, usually, some components remained unseparated near the start or finish line. Therefore Ruta graveolens is a very good model plant. Methanol and petroleum ether extract from its aerial parts were used to demonstrate the capabilities of the new device for gradient thin-layer chromatogram development. The development of gradient thin-layer chromatograms in the reversed-phase system in conventional horizontal chambers can be disrupted by problems associated with an excessive flux of the mobile phase to the surface of the adsorbent layer. This phenomenon is most likely caused by significant differences between the surface tension of the subsequent fractions of the mobile phase. An excessive flux of the mobile phase onto the surface of the adsorbent layer distorts the flow of the mobile phase. The described effect produces unreliable, and unrepeatable results, causing blurring and deformation of the substance zones. In the prototype device, the mobile phase solution is delivered onto the surface of the adsorbent layer with controlled velocity (by moving pipette driven by 3D machine). The delivery of the solvent to the adsorbent layer is equal to or lower than that of conventional development. Therefore chromatograms can be developed with optimal linear mobile phase velocity. Furthermore, under such conditions, there is no excess of eluent solution on the surface of the adsorbent layer so the higher performance of the chromatographic system can be obtained. Directly feeding the adsorbent layer with eluent also enables to perform convenient continuous gradient elution practically without the so-called gradient delay. In the study, unique fingerprints of methanol and petroleum ether extracts of Ruta graveolens aerial parts were obtained with stepwise gradient reversed-phase thin-layer chromatography. Obtained fingerprints under different chromatographic conditions will be compared. The advantages and disadvantages of the proposed approach to chromatogram development with controlled solvent velocity will be discussed.

**Keywords :** fingerprints, gradient thin-layer chromatography, reversed-phase TLC, Ruta graveolens
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