Optimum Mobile Phase Condition for Resolving Isoflavones by HCI Program

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The optimization of mobile phase condition for eight isoflavones was determined by the software of HCI program. The optimum composition of mobile phase for the separation of the eight isoflavones was obtained on the basis of resolutions and separation times. The elution profiles were calculated by the plate theory based on the equations of retention factor, $\ln k = A + BF + CF^2$, and F was the vol.% of acetonitrile with 0.1%acetic acid(AA). We modified the plate theory to calculate elution profile on both isocratic and gradient mode. From the some calculated results, using the step-gradient mode was recommended. The first mobile phase composition was water with 0.1% AA/acetonitrile with 0.1% AA, 88/12 vol.%, then after 15 min, the second composition of mobile phase was step-changed to 85/15 vol.%, then after 25 min, the third composition was step-changed to 73/27 vol.%, then after 39 min, it was changed to 65/35 vol.% and finally it was kept on the isocratic mode to the end of run time, 50 min. In the experimental conditions, the agreement between the experimental data and the calculated values was relatively good.