ABSTRACT

WULAN TRI WAHYUNI S. Optimization and Validation High Performance Liquid Chromatographic Fingerprint of Phyllanthus niruri L. Under direction of LATIFAH K. DARUSMAN and AJI HAMIM WIGENA

Split-plot mixture-mixture design has been applied for optimization of Phyllanthus niruri L. chromatographic fingerprint. The design applied for unreplicated and simultaneous optimization of extraction mixture and chromatographic mobile phase mixture. The whole-plot was extraction solvent contained varying proportion of methanol, ethyl acetate and dichloromethane in a simplex-centroid with axial design. The sub-plot was reversed phase chromatographic mobile phase in simplex-centroid design contained varying proportion of methanol, acetonitrile and acetonitrile:water (55:45 v/v). Each extract analyzed with seven mobile phase and monitored at 210, 225 and 254 nm. Ratio whole plot error to subplot error smaller than 0.4 ($\frac{\sigma_{wp}}{\sigma_{sp}} < 0.4$). Correlation between extraction solvent, chromatographic mobile phase and number of peak analyzed statistically by Ordinary Least Square (OLS) method. The root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) at 210, 225 and 254 nm respectively were 1,86341 and 4,00759; 2,22201 and 5,28394; 1,54367 and 2,26063. Optimum condition obtained when ethyl acetate extract eluted by acetonitrile:water (55:45 v/v) and monitored at 254 nm. Validation of optimum condition performed for precision and extract stability test parameter. Precision of retention time at optimum condition was excellent, percent relative standard deviation (%RSD) were 0,0698 % - 0,3006 %. Extract stability test examined after 2,5 and 5 hours storage at 25 °C with protection from light, peak area of each extract component changed in different level during the storage.

Keywords: optimization, validation, chromatographic fingerprint, Phyllanthus niruri L., split-plot mixture-mixture design.