

## INTISARI

Zat pewarna memainkan peran penting untuk kesan terbaik dari produk makanan terutama minuman bubuk. Salah satu warna yang diizinkan adalah TAR, meskipun konsentrasinya dibatasi. Karena pewarna yang mahal, industri makanan mengganti TAR dengan pewarna ilegal seperti AUO. Namun, AUO lebih beracun dan dapat menyebabkan masalah pernapasan, keracunan akut-kronis melalui kulit dan berpotensi karsinogenik. Oleh karena itu, kontrol kualitas memerlukan metode analitik di mana batas deteksi rendah, cepat dan tepat.

Analisis TAR dan AUO dalam minuman serbuk dilakukan menggunakan kromatografi cair kinerja tinggi (KCKT) dan spektroskopi FTIR yang dikombinasikan dengan kalibrasi multivariat *partial least square* (PLS). Optimasi metode KCKT dengan *Central-composite Design*, dengan *software Design Expert 10.1*. Metode optimum divalidasi sesuai parameter *International Conference of Harmonization* (ICH) dalam hal selektivitas, linieritas, presisi, akurasi, batas deteksi dan batas kuantitasi. Analisis kuantitatif secara spektroskopi FTIR menggunakan model kalibrasi yang diperoleh dengan memplot kadar *actual* yang diperoleh dengan KCKT dengan kadar prediksi FTIR-PLS dari seri sampel. Model divalidasi secara internal dan eksternal. Kriteria model kalibrasi dan validasi berupa nilai *root mean square error of calibration* (RMSEC), *root mean square error of cross validation* (RMSECV), *Root Mean Square Error of Predicted* (RMSEP) dan  $R^2$  mendekati 1.

Kondisi optimal KCKT hasil CCD adalah: laju alir 1,2 mL/menit, konsentrasi ACN 86% dan konsentrasi bufer amonium asetat 19 mM. Hasil validasi metode analisis baik ditunjukkan oleh metode selektif, nilai  $R^2 > 0,997$  pada uji linearitas TAR dan AUO. Sensitif ditandai dengan nilai LOD dan LOQ rendah. Nilai RSD pada penetapan presisi *intra-day* dan *inter-day* lebih rendah dari RSD Horwitz. Nilai akurasi TAR dan AUO pada *level* konsentrasi 80, 100 dan 120% berada di rentang 90-107%. Uji *robustness* menghasilkan nilai  $p < 0,0001$  (0,05%). Model kalibrasi yang diperoleh diukur pada bilangan gelombang 1730-1264  $\text{cm}^{-1}$ . Model kalibrasi mempunyai nilai koefisien korelasi ( $r$ ) baik yaitu 0,9437 (TAR) dan 0,9381 (AUO). Validasi metode analisis tartrazin dan auramin O dalam minuman serbuk menggunakan spektroskopi FTIR-kalibrasi multivariat PLS menunjukkan hasil yang baik dengan validasi internal teknik validasi silang *leave one out* (RMSEC dan RMSECV kecil serta  $R^2$  mendekati 1) dan tetapi tidak untuk validasi eksternal ( $R^2$  jauh dari 1 dan nilai RMSEP cukup besar).

**Kata kunci : tartrazin, auramin O, spektroskopi FTIR, kalibrasi multivariat PLS, KCKT**

## ABSTRACT

The coloring agents play an important role for the best impression of the food products especially the powder drinks. One of the permitted colors is TAR, although its concentration will be limited. Due to the expensive dyes, the food industries replace TAR with the illegal dyes such as AUO. However, AUO is more toxic and can cause respiratory problems, acute-chronic poisoning through the skin and potentially carcinogenic. Therefore, the quality control requires an analytical method in which a low detection limit, fast and precise.

Analysis of TAR and AUO in powder drinks was carried out using high performance liquid chromatography (HPLC) and FTIR spectroscopy in a combination of multivariate partial least square (PLS) calibration. HPLC method optimization with Central-Composite Design, Design Expert software 10.1. The optimum method is validated according to ICH parameters in terms of selectivity, linearity, precision, accuracy, detection limit and limit of quantitation. Quantitative analysis using FTIR spectroscopy used a calibration model obtained by plotting the actual levels of HPLC with prediction levels of FTIR-PLS from the sample series. The model is validated internally and externally. Validation criteria are root mean square error of calibration (RMSEC), root mean square error of cross validation (RMSECV), Root Mean Square Error of Predicted (RMSEP) and  $R^2$  approaching 1.

Optimal conditions for CCD HPLC are: flow rate of 1.2 mL/minute, ACN concentration of 86 % and ammonium acetate buffer concentration of 19 mM. The results of good analysis method validation are shown by the selective method, the value of  $R^2 > 0.997$  in the TAR and AUO linearity test. Sensitively characterized by LOD and low LOQ values. RSD values on intra-day and inter-day precision determination were lower than RSD Horwitz. The accuracy values of TAR and AUO at concentration levels of 80, 100 and 120 % are in the range of 90-107 %. Robustness test produces a value of  $p < 0.0001$  (0.05 %). The calibration model obtained is measured at wave number  $1730-1264 \text{ cm}^{-1}$ . The calibration model has a good correlation coefficient ( $r$ ) value of 0.9437 (TAR) and 0.9381 (AUO). Validation of the method of analysis of tartrazine and auramine O in powder drinks using multivariate PLS FTIR-calibration spectroscopy showed good results with internal validation leave one out cross validation techniques (small RMSEC and RMSECV and  $R^2$  approaching 1) and but not for external validation ( $R^2$  is far from 1 and the RMSEP value is quite large).

**Keywords: tartrazine, auramine O, FTIR spectroscopy, multivariate calibration PLS, HPLC**