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FULL PAPER

Analytical method validation of reversed phase HPLC for quantitative analysis of tartrazine and auramine o in powder drinks

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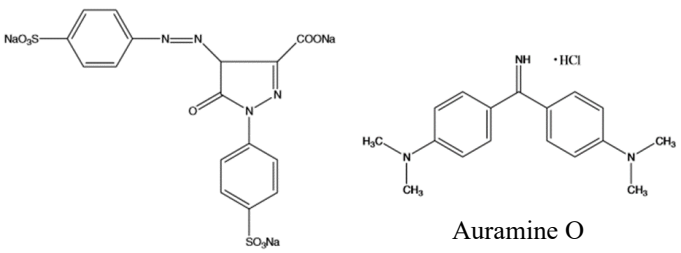
Abstract

This study was aimed to perform analytical method validation of high-performance liquid chromatography (HPLC) technique using photo-diode array detector for the simultaneous determination of Tartrazine (TAR) and Auramine O (AUO) in powder drink products. TAR and AUO were analysed using Waters Shield C_{18} column (250 mm x 4.6 mm i.d., 5 µm) using PDA detector at 300-650 nm. The mobile phase used was acetonitrile-ammonium acetate 19 mM (86:14 v/v) delivered isocratically at a flow rate of 1.2 mL/ min. The optimized HPLC condition was subjected to analytical method validation by assessing some performance characteristics as guided by International Conference on Harmonization (ICH). The method was linear over the studied concentration ranges with the coefficient of determination (R^2) of 0.999 and 0.997 for TAR and AUR with % intercept less than 2%, respectively. The developed method was sensitive with a limit of detection value of 0.0325 µg/mL and 0.1052 µg/mL for TAR and AUO, respectively. The method is also accurate and precise as indicated with acceptable recovery values of 99.0-100.7% for TAR and 102.1-106.5% for AUO with relative standard deviation (RSD) values lower than those required by Association of Official Analytical Chemists' (AOAC). The developed method is simple and can be used for routine analysis of TAR and AUO for quality assurance purposes of powder drinks.

1. Introduction

Natural and artificial food dyes are added to food ingredients in order to increase one's appetite and to trigger the consumption of food products. Synthetic dyes are more stable and less expensive than natural dyes, therefore, synthetic dyes are widely used in the food industry to lower the production costs (Khiralla *et al*., 2015; Weisz *et al*., 2018). Because the legal food colourants have fewer variations and have more expensive prices, there has been an addition or substitution of legally used colouring agents with illegal ones in food products to get economical profits. The synthetic dyes often have high toxicity and side effects, including teratogenic, carcinogenic and mutagenic effects which of course pose a high risk to consumers' health (Li *et al*., 2018). Tartrazine (TAR) and Auramine O (AUO) having chemical structures in Figure 1 are dyes used in the food products. TAR can be used in powder drinks product, while AUO is not allowed. Due to its similarity colour, TAR is frequently replaced by AUO (Tatebe *et al*., 2014; Bachalla, 2016).



Tartrazine Auramine O

Figure 1. The chemical structures of Tartrazine (TAR) and Auramine O (AUO)

TAR is one of the synthetic dyes used in the food industry with a certain maximum value limit. Hyperactive behaviour in children has been reported due to food consumption containing TAR (Stachowiak and Elliotta, 2017; Vidal *et al*., 2018). In addition, AUO is used in biological and industrial applications. The exposure of AUO in food and cosmetics for a long time

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has been associated with cancer and acute oral toxicity (Sabnis, 2010; Kim *et al*., 2019). Therefore, analytical methods for quantitative analysis of TAR and AUO must be developed in order to ensure food safety.

Several methods have been used for the analysis of TAR and AUO, namely thin layer chromatography (Li *et al*., 2014), HPLC with the detector of the photo-diode array (Minioti *et al*., 2007; Šuleková *et al*., 2016; Rejczak and Tuzimski, 2017), liquid chromatography-mass spectrometry (LCMS) (Li *et al*., 2014) and FTIR spectroscopy combined with multivariate data analysis (Karimi *et al*., 2016). However, most of this method was used for the analysis of TAR and AUR individually, and among our best knowledge, there is a limited publication reporting quantitative analysis of TAR and AUO simultaneously. Therefore, HPLC with PDA detector was validated for quantitative analysis of TAR and AUO in powder drink formulation in this study.

2. Materials and methods

2.1 Materials

Powder drink products were obtained from several markets around Yogyakarta. The reference standards of Tartrazine (TAR) and Auramine O (AUO) were acquired from the National Agency of Drug and Food Control (NADFC) of the Republic of Indonesia. All solvents used for the mobile phase were of HPLC grade and were obtained from E. Merck (Darmstadt, Germany). Aquabidest was obtained from Ikapharmindo (Indonesia).

2.2 Preparation of reference standards

For the preparation of the stock solution, an approximately of 50.00 mg of each TAR and AUO was accurately weighed using an analytical balance (Mettler Toledo MX5) with the sensitivity of 0.01 mg and was added into volumetric flask 50 mL and dissolved with aquabidest until volume to get solution with a concentration of 1000 µg/mL. This solution was then used for preparing the calibration curve.

2.3 Preparation of samples

An approximately of 1000.0 mg of powder drink products were accurately weighed using an analytical balance (Mettler Toledo MX5) with the sensitivity of 0.1 mg, and dissolved with aquabidest until 50.0 mL. Then, 1.0 mL aliquot from the standard stock solution was transferred to 10 mL volumetric flask and the volume was made up to the mark with aquabidest. The solution was filtered with Polytetrafluoroethylene (PTFE) 0.45 µm and injected into the HPLC system.

2.4 HPLC instrumentation

TAR and AUO were separated by Waters Shield C_{18} column (250 mm x 4.6 mm i.d., 5 µm) using Shimadzu LC 20AD chromatograph equipped with photodiode array (PDA) detector at 300-650 nm. The mobile phase used was acetonitrile-ammonium acetate buffer 19 mM (86: 14 v/v) delivered isocratically at a flow rate of 1.2 mL/min.

2.5 Validation of analytical method

Validation is an important component of quality assurance intended to fit the analytical purposes (Sharma *et al*., 2018; Nuvitasari *et al*., 2019). Some validation parameters tested include selectivity, linearity, sensitivity expressed by the limit of detection and limit of quantification, precision, accuracy and robustness as in guideline in International Conference on Harmonization (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005; Solanki *et al*., 2018).

2.6 Data analysis

All validation data (linear regression, relative standard deviation (RSD) and recovery) were expressed as mean ± standard deviation (SD) of mean and were calculated using Microsoft Excel (Microsoft Inc., USA).

3. Results and discussion

HPLC technique is the most popular analytical methods for the separation of dyes including TAR and AUO because of its accuracy, precision, sensitivity, time efficiency, low cost and robust (Kucharska and Grabka, 2010). The different polarity between TAR and AUO could be separated using a C_{18} column. Figure 2 exhibited the separation of TAR and AUO using the optimized condition. Both analytes (TAR and AUO) were well separated with resolution value (Rs) of 7.27 indicating that RP-HPLC was selective enough for the separation of both analytes. The system suitability test

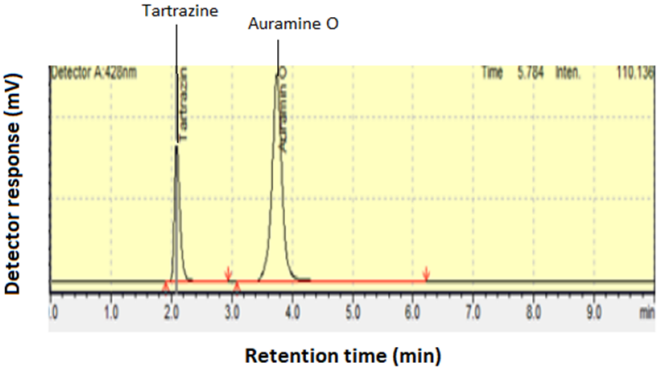

Figure 2. Separation of tartrazine (TAR) and Auramine O (AUO) using HPLC condition

indicated that RP-HPLC system was reliable for the quantitative analysis of TAR and AUO as indicated by relative standard deviation (RSD) values of retention times of TAR and AUO as well as peak areas of TAR and AUO were less than 2% (Sharma *et al*., 2018).

RP-HPLC method was then subjected to analytical method validation according to International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (2015) guidelines by evaluating some performance characteristics which include linearity and range, sensitivity as expressed by limit of detection (LoD) and limit of quantification (LoQ), precision expressed as RSD of repeatability and intermediate precision and accuracy expressed by the recovery of analytes added in the powder drink samples. The linearity of RP-HPLC was evaluated by preparing a series of standard solutions of TAR and AUO with certain concentration and correlating between the concentrations of both analytes (x-axis) and peak area of chromatograms (y-axis). The values of coefficient correlation (R-value) were higher than 0.999 which indicated that linear relationship existed between concentration and peak area at a certain concentration. The sensitivity of RP-HPLC with PDA detector for quantitative analysis of TAR and AUO was determined, and the obtained LoD values were 0.0325 µg/mL and 0.1052 µg/mL for TAR and AUO, respectively. In addition, LoQ values were calculated as: 3.33 x LoD values; and LoQ values reported were 0.1328 µg/mL and 0.3420 µg/mL for TAR and AUO, respectively.

The precision of RP-HPLC using a PDA detector was described by relative standard addition (RSD) values during repetition (intra-assay) and intermediate precision (inter-assay) using different analysis days. The precision of the analytical method is acceptable if RSD values obtained are lower than those in Horwitz's criteria (Gonzalez and Herrador, 2007; Miller and Miller, 2010). The RSD values from six replications of injecting TAR and AUO at concentration levels of 22.34 µg/mL (TAR) and 23.97 µg/mL (AUO) were 0.86% and 0.10%, respectively. In addition, RSD values for intermediate precision from different days were 0.83% and 0.12% for TAR and AUO, respectively. The maximum values of RSD Horwitz for analytes with a level of 23.0-24.0 µg/mL is 7.3%, therefore, all RSD values during intra-assay and intermediate precision (inter-assay) are lower than 7.3% (Gonzalez and Herrador, 2007), therefore, RP-HPLC method is precise for the quantitative analysis of TAR and AUO in powder drink products.

The accuracy of RP-HPLC was evaluated by recovery percentage using the standard addition method. In this case, reference standard solutions with known concentrations of TAR and AUO at three different concentrations (i.e. 80%, 100%, and 120% from target analytes) were added into blank powder drink samples (no detectable analytes). Tables 1 and 2 show the recovery percentages of TAR and AUO as analysed using RP-HPLC. The Association of Official Analytical Chemists (AOAC) set up that the recovery percentage of

Table 1. The recovery percentages of Tartrazine as analysed using reversed phase-high performance liquid chromatography

The levels of standard spiked into powder drink (µg/mg)	The concentration found (µg/mg)	The percentage recovery	Acceptance criteria (%)
0.774	0.769	99.3	80-110
0.775	0.768	99.2	80-110
0.774	0.766	99.0	80-110
1.16	1.12	96.5	80-110
1.161	1.12	96.4	80-110
1.161	1.109	95.5	80-110
1.548	1.558	100.6	80-110
1.547	1.548	100.1	80-110
1.547	1.542	99.7	80-110

Table 2. The recovery percentages of Auramine O as analysed using reversed phase-high performance liquid chromatography

The levels of standard spiked into powder drink (µg/mg)	The concentration found (µg/mg)	The percentage recovery	Acceptance criteria (%)
0.800	0.820	102.5	80-110
0.800	0.817	102.1	80-110
0.799	0.818	102.3	80-110
1.199	1.274	106.3	80-110
1.199	1.277	106.5	80-110
1.200	1.278	106.5	80-110
1.598	1.674	104.7	80-110
1.599	1.681	105.1	80-110
1.598	1.680	105.1	80-110

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analytes at level <100 ppm (µg/mg) was in the range of 80-110% (Gonzalez and Herrador, 2007), therefore, the recovery percentages of TAR and AUO obtained using RP-HPLC was acceptable. It was also stated that systematic errors could be negligible (Eka *et al*., 2012).

The robustness of the method developed was studied by changing the flow rate, the composition of the mobile phase and the concentration of the buffer. Changes in conditions were made intentionally in flow rates (± 0.2 mL/minute), the composition of organic solvents in the mobile phase (± 1 unit) and buffer concentration (± 5 units). The % RSD of the peak area and retention time is calculated by ANOVA for each evaluation during condition changes. The ANOVA results indicated that p-values were less than 0.05, therefore, the change of HPLC conditions from the optimized one is significantly different.

4. Conclusion

RP-HPLC using C_{18} column with PDA detector has been successfully validated for the quantitative analysis of TAR and AUO in powder drink samples. The developed method was accurate and precise as indicated by acceptable values of relative standard deviation (RSD) and recovery percentage. The validated RP-HPLC method was simple with no excessive sample preparation and suitable to be used for routine analysis of dyes (TAR and AUO) in powder drink samples.

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