

Simultaneous determination of tartrazine and auramine O using FTIR spectroscopy and partial least square

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Simultaneous determination of tartrazine and auramine O using FTIR spectroscopy and partial least square

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Abstract

Nowadays, the use of colouring agents are needed to increase customers' acceptability of foods. However, the development of food technology averts a big opportunity to use prohibited food colouring agents. Tartrazine (TAR) and Auramine O (AO) are two colouring agents may be used as food colorants. TAR is allowed, while AO is not allowed to be used in food colorants, respectively. For this study, the goal was to validate and apply Fourier transform infrared (FTIR) combined with multivariate calibrations for quantitative analysis of TAR and AO in beverages. An attenuated total reflectance FTIR scan of samples was performed at wavenumbers ranging from 4000-650 cm^{-1} . Quantitative analysis using FTIR spectroscopy was made by plotting the actual levels of TAR and AO obtained using HPLC and the prediction levels facilitated by partial least square (PLS) from the sample series. Internal cross-validation is performed on the model using the leave-one-out method. The root mean square error of prediction (RMSEP), the root mean square error of cross validation (RMSECV), the root mean square error of calibration (RMSEC), as well as the coefficient of determination (R^2) become validation criteria. The results exhibited that calibration model was gained using variables of FTIR spectra at wavenumbers of 1730-1264 cm^{-1} . Correlation coefficients (r) of 0.9437 (TAR) and 0.9381 (FTIR) are present in the calibration model for the correlation of actual and predicted values. The method for analyzing tartrazine and auramine O in powdered beverages was validated using multivariate PLS FTIR calibration spectroscopy. FTIR spectroscopy could be further developed as an alternative technique for analysis of TAR and AO in beverages.

1. Introduction

Food additives are typically added to food and drinks to give specific function, and one of them are food colorants. Based on the sources, food colorants are grouped into two groups, namely natural and synthetic (Griffiths, 2020; Kendrick, 2016). Either synthetic or natural food colorants are widely used in different food and drink products; however, in the market, the synthetic colorants dominate because of its availability and easy application methods (Nambela *et al.*, 2020). In addition, synthetic dyes are less expensive and more stable than natural dyes, therefore, synthetic dyes are widely used in the food and beverage industries in order to lower the production costs (Martins *et al.*, 2016).

Due to the price difference between allowed and non-allowed food colorants, some unethical producers substitute allowed colorants with unallowable ones to get economical profits. The substitution acts could be very problematic in health-related issues, due to its toxicity (Amchova *et al.*, 2015). The synthetic dyes often have high toxicity as well as side effects, such as teratogenic, carcinogenic, as well as mutagenic effects which of course pose high risk to consumers' health (Martins *et al.*, 2016). One of the food colorants used is tartrazine (TAR), and sometimes illegal colorants such as auramine O (AO) were used. Therefore, several analytical methods are reviewed, proposed, and applied for quality control of both food colorants (synthetic and natural) to assure its safety (Yamjala *et al.*, 2016; Ntrallou *et al.*, 2020).

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Some side effects have been reported due to the prolonged use of TAR including hyperactive behavior in children (Lipskikh *et al.*, 2020), while AO is reported to cause cancer (Combes and Haveland-Smith, 1982). Numerous analytical methods have been reported for analysis of TAR and AO, mostly based on chromatographic techniques including paper chromatography (Gharaghani *et al.*, 2020), liquid chromatography with several detectors (Tatebe *et al.*, 2014) with several systems (Sha *et al.*, 2014), and thin layer chromatography (Soponar *et al.*, 2008). The chromatographic methods are complex and requiring skillful analyst, hence several simple and rapid techniques based on FTIR spectroscopy is developed for analysis of colorants. FTIR spectroscopy is vibrational spectroscopy widely applied for analysis of food components including colorants (Gillard *et al.*, 1994). Hence, in this study, FTIR spectroscopy combined with multivariate calibration of partial least square has been proposed for analysis of TAR and AO in powdered drink samples.

2. Materials and methods

2.1 Materials

Tartrazine (CI 19140, Control Number: 110397) and auramine O (CI 41000, Control Number: B0114315) reference standards were obtained from Badan Pengawas Obat dan Makanan, Indonesia. The powdered beverage samples were gained from local market in Yogyakarta. HPLC mobile phase, obtained from Merck (Darmstadt, Germany), were of high-performance liquid chromatography (HPLC) grade. Distilled water was obtained from Ikapharmindo (Indonesia).

2.2. Analysis of tartrazine and auramine O using HPLC

The actual values of TAR and AO to be correlated with the predicted values in FTIR spectroscopy analysis in powdered drink samples were analyzed using HPLC. HPLC condition was previously optimized with Central Composite Design (Lestari *et al.*, 2019). The instrument used was HPLC chromatograph (Shimadzu LC-20AD, Japan) connected with detector photo-diode array (PDA) at the wavelength of 300-650 nm. Separation of TAR and AO was carried out with reversed phase C18 column (XBridge HPLC RP 18 250 mm × 4.6 mm i.d., 5 μm). Water-acetonitrile (14-86%) mobile phase was used, containing ammonium acetate buffer at a concentration of 19-21 mmol, and delivered isocratically at a flow rate of 1.2 mL/min. Millipore 0.45 μm filters were employed to obtain essential solutions to be further administered into HPLC systems.

2.3 Analysis of samples using FTIR spectroscopy

FTIR spectral measurement was carried out as in Prabaningdyah *et al.* (2018). The samples of powdered drinks were directly placed in the diamond crystal of attenuated total reflectance (SMART iTR™-ATR) with instrument of FTIR spectrophotometer (Thermo Scientific Nicolet iS10, USA) equipped with software of OMNIC for spectral processing as well as TQ Analyst for chemometrics (multivariate calibration) analysis. From 32 scans in the infrared range (4000-650 cm⁻¹) at a 16-cm resolution, the FTIR spectra were obtained. Absorption values were used to measure the TAR and AO concentrations because of Lambert-Beer law, subsequently, the air spectra were exercised to construct a background.

2.4 Data analysis

The HPLC and FTIR predicted values of TAR and AO were employed to calculate a multivariate calibration of partial least squares (PLS). Analyzing built models was carried out using R-value, root mean prediction error (RMSEP), as well as root mean square calibration error (RMSEC), all of which are statistical parameters (Miller and Miller, 2010). All statistics calculations were carried out using TQ Analyst software version 9 included in FTIR spectrophotometer.

3. Results and discussion

HPLC condition for analysis of TAR and AO in powdered drink samples was previously optimized with Central Composite Design (CCD) experimental design by Lestari *et al.* (2019), and this condition has been validated and meet the required requirements. The performance characteristic evaluated include selectivity, linearity and range, specificity, sensitivity as expressed as limit of detection (LoD) and limit of detection (LoQ), precision, accuracy and robustness. All parameters meet the acceptance criteria as detailed in Eurachem and International Standardization of Organization 17025 related to analytical method validation. Figure 1 reveals HPLC chromatogram indicating good separation between TAR and AO with resolution (Rs) value > 2.0. The retention times (tR) of TAR and AO were an approximately 2.08 mins and 3.74 mins, respectively. The validated HPLC method was reference method due to its capability to provide qualitative and quantitative analyses. Based on the validated results, HPLC was employed to quantify the levels of TAR and AO. The HPLC results were designated as actual values to be modelled with FTIR spectroscopy and multivariate calibration and were compiled in Table 1.

Figure 2 reveals FTIR spectra of powdered drink

Table 1. The contents of TAR and AO in powdered drink samples as determined using HPLC and predicted by FTIR spectroscopy combined with partial least square (FTIR-PLS).

Samples no	TAR		AO	
	Actual concentrations as determined by HPLC (µg/g)	Predicted concentration as determined by FTIR-PLS (µg/g)	Actual concentrations as determined by HPLC (µg/g)	Predicted concentration as determined by FTIR-PLS (µg/g)
1	16.72	16.35	0.74	1.54
2	15.72	14.85	2.61	2.96
3	15.32	14.55	2.97	3.06
4	13.43	14.03	3.10	3.59
5	14.09	14.13	3.84	3.50
6	14.28	14.25	3.68	3.89
7	13.90	13.67	4.39	4.68
8	13.32	13.60	4.62	4.78
9	13.38	13.64	5.00	4.86
10	13.13	13.01	5.70	5.67
11	12.89	13.15	5.91	5.53
12	12.11	13.08	6.20	5.66
13	12.24	13.23	7.11	5.23
14	12.42	12.83	7.45	5.95
15	11.26	10.72	7.22	7.37
16	11.77	10.56	7.40	7.63
17	10.15	9.69	7.76	8.19
18	10.22	9.89	6.88	8.10
19	8.07	9.78	6.97	7.82
20	9.74	9.30	8.85	8.35
Equation	$y = 0.8898 + 1.4074$		$y = 0.8829 + 0.6327$	
R	0.9437		0.9381	
Slope	0.8898		0.8829	
Intercept	1.4074		0.6327	

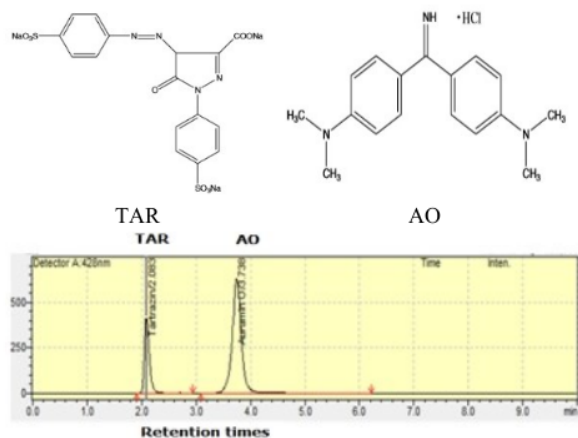


Figure 1. The separation of TAR and AO along with chemical structures using HPLC with C18 column.

samples containing TAR and AO at different concentrations at wavenumbers of 4000-650 cm^{-1} . Each peak and shoulders in FTIR spectra indicated the functional groups of components present in studied samples. Peaks at wavenumbers ($1/\lambda$) 3315 cm^{-1} indicated the presence of hydrogen bonding of O-H, peak at $1/\lambda$ 1644 cm^{-1} is coming from stretching vibration of functional group of C=C, peak at $1/\lambda$ 1417

cm^{-1} originated from stretching vibration of N=N, peak at $1/\lambda$ 1340 cm^{-1} corresponded to functional group of C-O, while S=O was indicated by the presence of peak at 1118 cm^{-1} . Peak at $1/\lambda$ 1036 cm^{-1} indicated the stretching vibration of C-OH and peak 1741 cm^{-1} was coming from stretching vibration of carbonyl group (C=O) (Rohman *et al.*, 2014). Therefore, FTIR spectra confirmed the presence of TAR and AO due to the agreement of functional groups in FTIR spectra with those in TAR and AO.

For making the calibration models, a series of powdered drink samples was preparing by spiking TAR and AO in samples with actual concentration were determined by HPLC. Partial least square (PLS) calibration was used to assist the correlation of actual values and FTIR predicted values. PLS is inverse calibration commonly used for complex analytes in the mixture (Muttaqien *et al.*, 2016). Some optimization in terms of the wavenumber region are capable of optimum modelling by giving the highest R^2 and lowest errors in calibration. Table 2 compiled the statistical performances during calibration modelling of TAR and AO in terms of R^2 used for correlation indication, RMSEC used for

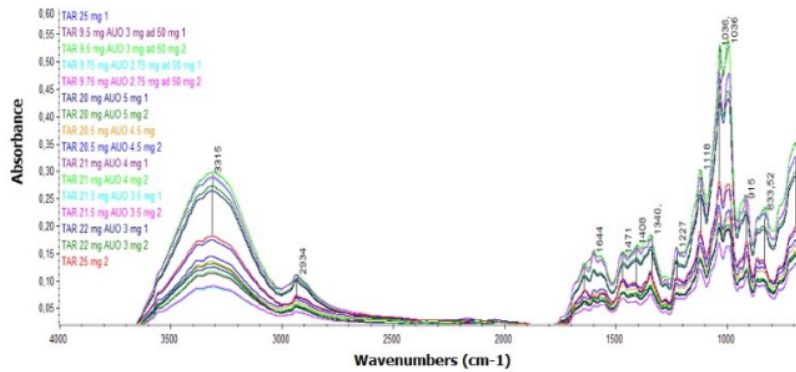


Figure 2. FTIR spectra of powdered drink samples containing TAR and AO at different concentrations of TAR and AO at wavenumbers of 4000-650 cm^{-1} using attenuated total reflectance.

Table 2. The statistical performances during calibration modelling of TAR and AO in powdered drink samples.

Wavenumbers region	Factor	TAR				AO			
		Calibration		Validation		Calibration		Validation	
		RMSEC	R ²	RMSEP	R ²	RMSEC	R ²	RMSEP	R ²
3999-650	2	0.5170	0.9483	1.4000	0.9112	0.5460	0.9524	1.3900	0.9395
1500-650	2	0.7050	0.9456	1.2200	0.7992	0.6740	0.9404	1.1600	0.8946
4000-1500	2	0.5960	0.9308	1.6000	0.8926	0.7500	0.9079	0.9950	0.9791
960-650	2	0.5540	0.9404	1.4500	0.9090	0.7310	0.9127	1.1000	0.9756
1264-960	2	0.7060	0.9012	1.6400	0.8640	0.5200	0.9569	1.8000	0.8632
*1730-1264	2	0.4930	0.9531	1.280	0.9392	0.6770	0.9256	0.8820	0.9922

*The wavenumbers region used.

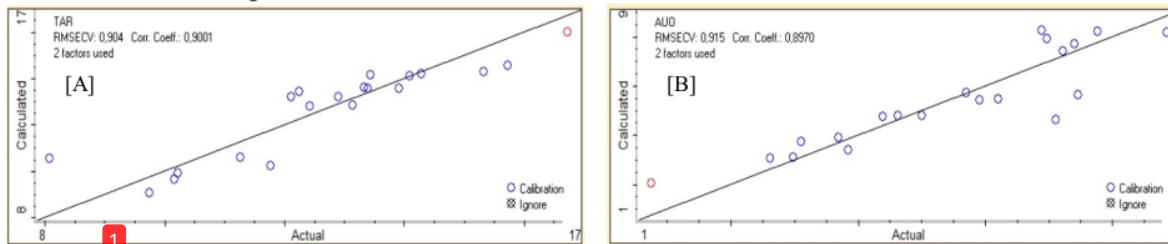


Figure 3. The correlation between actual values (x-axis) of TAR [A] and AO [B] and FTIR predicted values (y-axis) in cross validation using leave one out technique.

indication of calibration error, and RMSEP used as error indication in validation/prediction. The higher R² as well as the lower RMSEC and RMSEP indicated the better calibration model. Calibration models were built using absorbance values in the 1730-1264 cm^{-1} range. TAR and AO had correlation coefficients of 0.9437 and 0.9381, respectively. In the case of both TAR and AO, RMSEC was found to be 0.493% and 0.677%. The high R² values in calibration model indicated that the developed model provides high accuracy, while the low values of RMSEC exhibited that the model is precise enough, therefore the calibration model is reliable for the prediction of unknown samples (Lestari et al., 2017).

The calibration model obtained was validated using leave one out technique. During cross-validation using leave-one-out technique, one calibration sample from a set of calibration samples is removed, and the remaining calibration samples were used for constructing a new calibration model. This new model was subsequently

used to predict the value of the removed one. This process was repeated until all calibration samples were removed one by one, and the difference between actual values and predicted values were calculated and expressed as root mean standard error of cross validation (SECV) (Rohman et al., 2014). The lowest of RMSECV and the highest of R² elaborated that the obtained calibration model is the optimal calibration model chosen (Sadegaski et al., 2021). Figure 3 reveals the correlation of actual values (x-axis) of TAR and AO with FTIR predicted values (y-axis) in cross validation using leave one out technique. During cross validation, R² and RMSECV values of 0.81 and 0.90% for TAR as well as 0.80 and 0.91% for AO. Therefore, this result is quite good according to internal validation criteria.

4. Conclusion

Analysis of tartrazine and auramine O using FTIR spectroscopy and partial square has been proposed as an

alternative to HPLC. By optimizing the wavenumbers region that is able to provide the highest accuracy and lowest errors, the absorbance values at wavenumbers of 1730-1264 cm^{-1} were exploited for the modelling between levels of TAR and AO obtained using HPLC and levels of TAR and AO using FTIR spectroscopy. The developed method is rapid without any extensive sampling preparation.

Conflict of interest

The authors declare no conflict of interest.

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