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ARTICLE

The Determination of Ethanol Levels in Facial Freshener Using the NIR Spectroscopy and Chemometric Method

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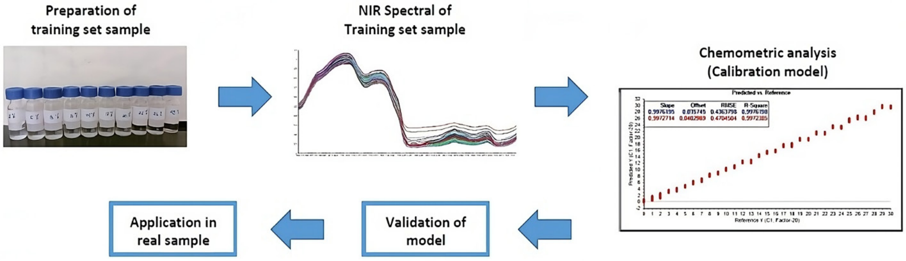

A facial freshener, also known as toner, is a cosmetic product that is commonly used to invigorate the face after a busy day. Ethanol serves as a key component in toner, serving multiple purposes such as being a solvent, preservative, and antimicrobial agent. However, it's important to note that toner formulated for normal skin types typically contain ethanol in small concentrations, adhering to a limit of not more than 10%. Therefore, this study aims to determine ethanol levels in toner using the NIR spectroscopy and chemometric techniques. The NIR spectra of the simulated samples were correlated with ethanol concentration using chemometric calibration model. The calibration models used were partial least square (PLS), principal component regression (PCR), and support vector regression (SVR). The calibration model was validated by leave one out cross validation (LOOCV) as well as the external validation, and the precision and accuracy of the method was evaluated. Among the calibration models, the PLS model exhibited the best performance, yielding an impressive R^2 0.9976; with an RMSEC value of 0.4364 and RMSECV value of 0.4704. The internal validation yield R^2 value more than 0.99 and RMSE of less than 0,4198. Furthermore, external validation showed the R^2 and RMSEP value of 0.989 and 0.920 respectively. The %recovery and RSD value were 101.2% and 0.129%. Comparing ethanol measurements obtained through the NIR chemometric method with those obtained using gas chromatography as the reference method, no significant difference was observed at a 95% confidence levels, as indicated by a significance value of 0.231.

Keywords: NIR spectroscopy, chemometric, facial freshener, ethanol, toner

INTRODUCTION

Cosmetics refer to substances or mixtures used externally on the human body, such as the skin, scalp hair, nails and the outer parts. They serve the purpose of cleaning, maintaining, enhancing attractiveness, and protecting the body, excluding the medicinal applications.[1] One of facial cosmetics that is commonly

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used by women, especially Indonesia, is a face freshener (toner). Toner is used to cleanse the face after daily activities, refresh the pH balance of facial skin, and achieve optimal equilibrium.[2] They are typically composed of various natural ingredients. For normal skin types, toner often include moisturizing agents to restore hydration after using facial cleansers.[3] In the formulation of toner, ethanol can be used as a solvent, preservative and antimicrobial. It helps in dissolving active ingredients or substances that are insoluble in water.[4] Toner are usually recommended for individuals with normal and oily skin types.[5] The concentration of ethanol used in toner for normal skin is limited to no more than 10%, as it provides the desired therapeutic effect.[6]

According to previous studies, the determination of ethanol content was carried out using several method. In a study by Almeida et al (2021), the determination of ethanol and fragrance content in cosmetics was carried out using gas chromatography method.[7] According to the study by Pinu et al (2017), ethanol content in fermented foods and beverages were determined using the gas chromatography method.[8] Valentina et al (2010) examined the determination of ethanol content in branded foods using chemometric NIR (Near Infrared) spectroscopy method.[9] Mmaabo (2022) examined the determination of ethanol content in various types of alcoholic beverages using the gas chromatography method.[10] Rebecca's (2021) explored the determination of ethanol content in beer using gas chromatography method.[11] However, no study has reported the determination of ethanol content in cosmetic samples, especially toner, using either gas chromatography or NIR spectroscopy method. The gas chromatography method offers numerous benefits, including high sensitivity, but it has the drawback of being time-consuming and requiring the preparation of a chemical mixture. In this study, the determination of ethanol content was carried out using the NIR spectroscopy method. This method is not only applicable to cosmetic toner samples but also enables the assessment of alcohol content in beverages like beer.[12] One of the major advantages of the NIR spectroscopy is its ability to provide quick analysis results without causing pollution, as it solely relies on simple preparations without the need for chemical compounds.[13] However, the NIR spectroscopy has drawbacks, including spectra that require further interpretation due to spectrum overlap and low sensitivity. To overcome these challenges, chemometric method are employed in conjunction with the NIR spectroscopy to rapidly identify and analyze spectra.[14] In this study, toner simulation samples with ethanol concentrations ranging from 0% to 30% were used for the NIR method.

This study aims to determine ethanol content in toner using the NIR chemometric method and compare the results with gas chromatography as a reference method in order to ascertain if there is a significant difference between the NIR spectroscopy method and gas chromatography.

MATERIALS AND METHOD

Materials

In this study, the materials used were ethanol (Merck), n-butanol (Merck), cucumber extract, lemon extract, tween 80 (GBM), propylene glycol (Brataco), NaOH (Merck), nipagin (GBM), nipasol (GBM), lemon perfume (GBM), aquabidest (Wida). The real sampels of toner were purchased from the market in Jember, East Java, Indonesia.

Instrumentation

The tool used were the NIR Spectroscopy (Brimrose Luminar 3070), gas chromatography (GC-FID Thermo Scientific-Trace 1300), Software The Unscrambler X 10.4 (Oslo, Norway), pH meter, and laboratory glassware.

Sample Simulation Preparation

Sample simulation preparation involved the creation of a sample training set and a test set. The formulation ingredients, including cucumber extract, lemon extract, tween 80, propylene glycol, NaOH, nipagin, nipasol, and lemon perfume, were mixed together. Ethanol and aquabidest were then added to achieve various concentrations in the training set samples (ranging from 0% to 30% v/v) and the test set samples (ranging from 2.5% to 29.5% v/v).

Sample Measurement Using the NIR Spectroscopy

Sample measurements were carried out using the Brimrose NIR spectroscopy instrument. Furthermore, 200 µL of each sample was placed on the sample compartment using a micropipette. Each simulation sample was scanned in the range of 850-2000 nm. The measurements were replicated three times, with 10 shooted in each replication. The NIR spectroscopy used consisted of a 3070 brimrose luminar spectrometer with a sampling area measuring 5 x 3 mm and assisted by a computer set. The NIR Spectroscopy technique was based on measuring the light reflected by a sample. The NIR spectrum was obtained by converting the energy transferred from the radiation into mechanical energy through the movement of atoms and chemical bonds within molecules. Initially, the sample was exposed to a light source at a wavelength between 780-2500 nm and the light reflected or transmitted by the sample collected on the detector which was then converted into a spectrum.

Model Calibration and Validation

For model calibration and validation, the NIR spectras obtained were analysed by The Unscrambler X 10.4. The multivariate analysis used for calibration model were partial least square (PLS), principal component regression (PCR), and support vector regression (SVR).[15] The calibration models (PLS, PCR, and SVR) were formed by correlating the transmittance of the NIR spectra with ethanol concentration. The selected model was validated using leave one out cross validation (LOOCV) using 30 concentation samples training set and external validation using 10 concentration samples test set.[16] LOOCV was evaluated by remove one set of data in training set and the remaining data were used to form new calibration model. The precision and accuracy of the method was evaluated based on BP 2022.[17]

Real Sample Application

The selected calibration model that has been validated was applied to determine ethanol content in the commercial sample. For each commercial sample, 200 µL was placed on the NIR spectroscopy sample compartment and scanned three times for replicability. The real samples analyzed were five samples of toner containing ethanol purchased from the market in Jember, Indonesia.

The determination of ethanol by gas chromatography and validation method

To prepare the standard ethanol solution, ethanol was diluted in aquabidest (distilled water) to achieve a concentration range of 2-29% ethanol, with 1.0% n-butanol as the internal standard.[18] Real sample preparation involved adding 0.99 mL of the sample to 0.01 mL of n-butanol. The real samples were then subjected to gas chromatography analysis with three replicates. Subsequently, ethanol content was analyzed using the gas chromatography method with a detector temperature of 300 °C and an injector temperature of 200 °C, and the analysis was carried out by directly injecting 1 µL of sample in split mode with a split flow ratio and split ratio of 1:143. The initial temperature on the heater (oven) was set at 40 °C and held for the first 1 minute, after which it was increased by 10 °C per minute until reaching 70 °C. Subsequently, the temperature was increased at a rate of 70 °C per minute until reaching the final limit temperature of 200 °C, which took 6 minutes. The pressure in the carrier gas was maintained on the column at 60 kPa. The gas flux used for the detector was 350 mL per minute for synthetic gas and 35 mL per minute for hydrogen. Precision and accuracy were evaluated using three levels of simulated samples and replicated three times.

To compare ethanol levels in the real samples determined using the NIR chemometric and gas chromatography, a paired *t*-test was performed on the samples. The significance levels (p-value) for the test was set at 0.05 (two-tailed).

RESULTS AND DISCUSSION

The simulation sample consists of 31 samples calibration set with a concentration range of 0%-30% v/v and 10 samples test set with a concentration range of 2.5%-29.5% v/v. The concentration range was wider than concentrations commonly used in commercial toner (1% - 10% v/v). The wider the range of calibration set sample used the better the calibration model obtained.

The spectra of ethanol, simulated samples of 0%, 10% ethanol concentrations and commercial samples are shown in Figure 1. Ethanol spectrum, simulated samples and commercial samples has the same pattern, only the transmittance intensity is different as shown in Figure 1.

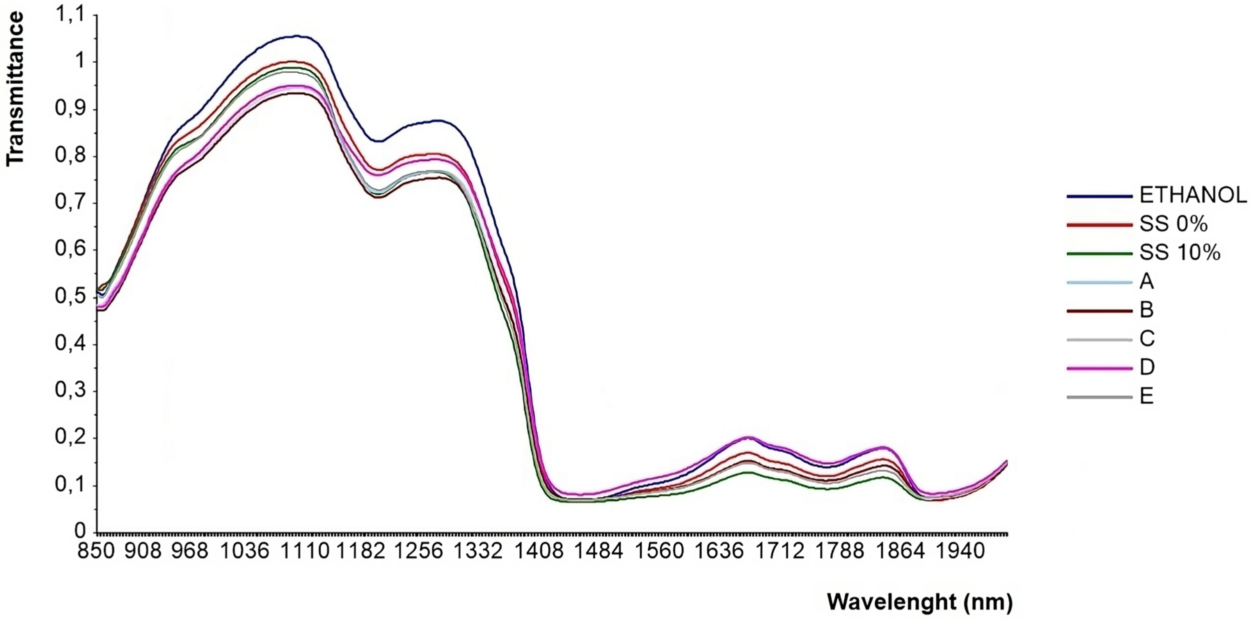

Figure 1. IR spectra of ethanol, simulation sample with ethanol concentration 0%, 10% (SS 0%, SS 10%) and commercial sample toner (A, B, C, D, E).

The calibration model for the NIR method was established using spectral data from a training sample set comprising 750 data points. Each concentstins in the training set samples was scanned using the NIR spectroscopy, resulting in 25 spectrum data for each concentration. In total, there were 30 concentrations, which produced 750 spectrum data used to build a calibration model. Among the calibration model tested, the best performance was achieved by the PLS using one latent variable with R^2 0.9976; RMSEC 0.4364 and RMSECV 0.4704 (Table I). The R^2 of internal validation (LOOCV) was more than 0.99 and RMSE less than 0,4198. When evaluating the calibration models, the key figures of merit considered were R^2, RMSE and RMSECV.[19] A higher R^2 value closer to 1 indicates a better calibration model, as it signifies a stronger correlation between the NIR spectra and ethanol concentrations. A smaller RMSE and RMSECV value suggest a better model calibration, as they indicate lower average prediction errors and better predictive performance.

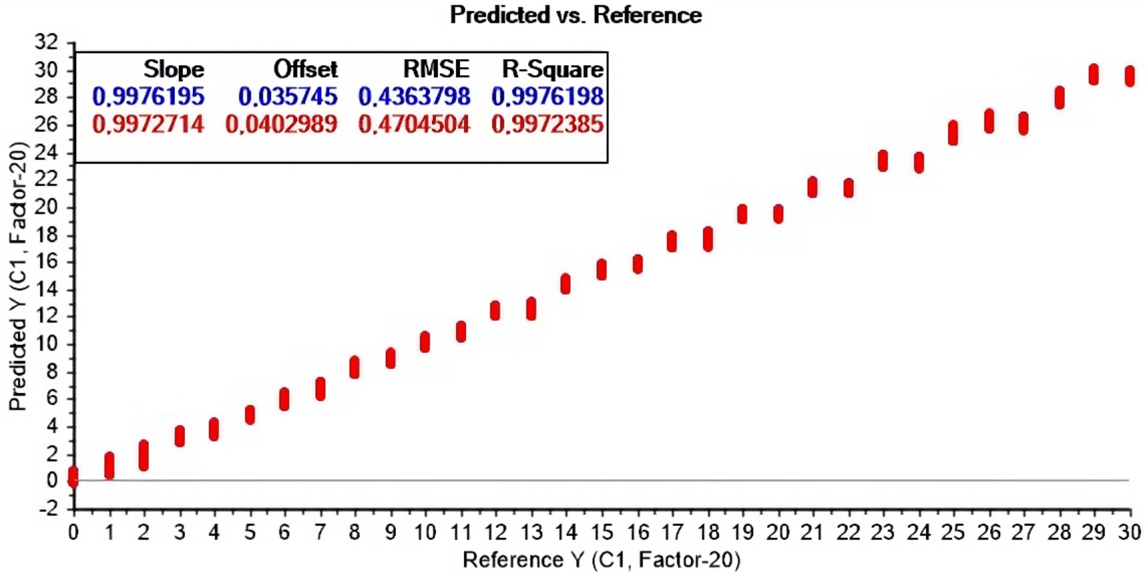

Figure 2. Graph of variations in the RMSECV values of the calibration samples in the PLS model.

Table I. The results of the formation of the calibration model

No	Model		RMSE	R^2
1.	PLS	Calibration Validation	0.4364 0.4704	0.9976 0.9972
2.	PCR	Calibration Validation	0.6909 0.7081	0.9940 0.9937
3.	SVR	Calibration Validation	0.4199 0.4194	0.9959 0.9959

The PLS model was selected as the optimal calibration model because it demonstrated an R^2 value of 0.9976, which is very close to 1. This high R^2 value indicates an excellent fit of the model to the data, suggesting a strong correlation between the NIR spectra and ethanol concentrations. Additionally, the PLS model exhibited a small RMSE value of 0.4364, which is below the threshold of 1.5. The RMSE value measures the average prediction error of the calibration model, and a smaller value indicates better accuracy in predicting ethanol concentrations. The selected PLS model satisfied the requirements for a good calibration model, as it had an R^2 value close to 1 and an RMSE value below 1.5. These criteria ensure that the model is capable of accurately predicting ethanol levels in toner samples based on the NIR spectra.[20]

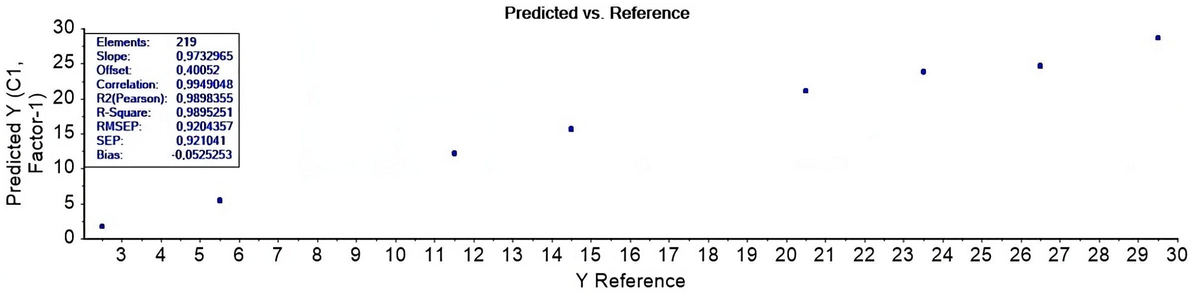

Figure 3. External validation of the PLS model.

The comparison method has been validated with the results of the parameter assessment of each validation stage listed in Table II.

Table II. Method validation results

Validation Parameters	**GC method**	**NIRS method (PLS model)**
Linearity		
Linierity range (%v/v)	2 – 29	0 – 30
Correlation coefficient (r)	0.999	-
Correlation coefficient (R^2)	-	0.989
Coefficient of variation (*Vx0*)	2.511%	
RMSEP	-	0.920
LOD	1.70 µg mL^{-1}	-
LOQ	5.12 µg mL^{-1}	-
Accuracy (%recovery, n=3x3)	101.9%	101.2%
Precision (RSD, n=3x3)	0.197%	0.129%

The results in Table II, show that GC method have the correlation coefficient of 0.999 and the coefficient of variation of 2.511%. This means that this method meets the requirements of linearity, the correlation coefficient (r) ≥ 0.99, and the function variation coefficient (Vx0) < 5%. The % recovery and RSD value were 101.9% and 0.197%, respectively. These results meet the accuracy and precision requirements that were % recovery within 95.0-105.0% and RSD value less than 2%.[21] For the NIR spectroscopy method, the external validation showed good results. The R^2 and RMSEP value using one of latent variable were 0.989 and 0.920 and the % recovery and a RSD value were 101.2% and 0.129%, respectively (Figure 3).

The commercial sample toner used were five samples with sample codes A, B, C, D and E. The results of ethanol levels in commercial samples are listed in Table III.

Table III. Results of the determination of ethanol content in commercial samples

Sample code	**Ethanol content (%v/v) ± SD (n=3)**	
	NIR spectroscopy	**Gas chromatography**
A	15.548 ± 0.032	15.212 ± 0.128
B	7.669 ± 0.009	7.481± 0.136
C	12.169 ± 0.012	12.131± 0.159
D	5.404 ± 0.012	5.468± 0.141
E	12.121 ± 0.019	12.114± 0.341

The comparison of ethanol levels in toner was conducted using the NIR-chemometric method and gas chromatography through a paired sample *t*-test. In order to proceed with the analysis, it was necessary to ensure that the data met the assumption of normal distribution ($p > 0.005$).[22] To assess the normality of the variables, a Kolmogorov-Smirnov test was performed using the IBM SPSS program. The normality test results obtained from the NIR method and gas chromatography were 0.702 and 0.615 respectively, the condition for normally distributed data is significant if the value is > 0.05. These data met the requirements, meaning both method were normally distributed and suggest the paired sample *t*-test could be continued. The paired *t*-test yielded a significant value of 0.231 with a 95% confidence levels. The results of the comparison obtained are said to be meaningful if the p value (sig.) in the 2 groups is> 0.05 with a degree of confidence reaching 95%, meaning that there is no significant difference between the two method.

CONCLUSION

In conclusion, ethanol content in toner sample was successfully determined using the NIR spectroscopy and chemometric method using PLS model with R^2 0.9976; RMSEC 0.4364 and RMSECV 0.4704. The PLS calibration method exhibited favorable results with an R^2 value of 0.9976, RMSEC of 0.4364, and RMSECV of 0.4704. The validation tests also met the requirements, demonstrating an R^2 value exceeding 0.99 and an RMSE below 0.4198. External validation showed an R^2 and RMSEP of 0.989 and 0.920. The % recovery and RSD value were 101.2% and 0.129%, respectively. There was no significant difference between ethanol content obtained from the NIRS-chemometric method and the gas chromatography method, as confirmed by the paired sample *t*-test with a significance value of 0.231 (> 0.05). Therefore, the NIRS-chemometric method can be considered reliable, relatively fast, easy and simple method for determining ethanol content in toner.

Conflicts of interest

There are no conflicts to declare.

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