ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2021.13.01.105

Research Article

Ultraviolet Spectroscopy Combined With Multivariate Calibration for Analysing Caffeine, Nicotinamide and Pyridoxine In Simulated Energy Drinks

MICHAEL RAHARJA GANI[1]*, FLORENTINUS DIKA OCTA RISWANTO[2]
[1,2]Department of Pharmacy, Faculty of Pharmacy, Sanata Dharma University, Campus III Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta 55284, Indonesia.
*Corresponding Author
Email ID: mr_gani@usd.ac.id
Received: 18.08.20, Revised: 18.09.20, Accepted: 18.10.20

ABSTRACT
Energy drinks, popular supplements containing caffeine and some water-soluble vitamins such as nicotinamide and pyridoxine are widely consumed and it is important to control and to maintain the nutritional adequacy of energy drinks. Therefore, it is necessary to develop a fast, simple and cost-effective means of analysing the caffeine, nicotinamide and pyridoxine content in energy drinks. Standard solutions of caffeine, nicotinamide, pyridoxine and the simulated energy drink samples containing all standard solutions were formulated and applied for TLC-densitometry and ultraviolet spectroscopy. We obtained original, first derivative, second derivative, standard normal variate and Savitzky-Golay smoothing spectra using ultraviolet spectral pre-processing. Two multivariate calibration techniques namely partial least squares and principal component regression were applied to all spectra in order to obtain appropriate model for each compound. We successfully developed an ultraviolet spectroscopy method coupled with multivariate calibration. The multivariate calibration models for caffeine, nicotinamide and pyridoxine were partial least square with original spectra, principal component regression with original spectra and principal component regression with Savitzky-Golay smoothing spectra, respectively. The linear equation model used for predicting the caffeine, nicotinamide and pyridoxine content was $y = 1.068x - 0.889$ ($R^2 = 0.988$), $y = 1.237x - 1.923$ ($R^2 = 0.975$), and $y = 1.150x - 0.722$ ($R^2 = 0.977$), respectively. The caffeine, nicotinamide and pyridoxine content in the simulated energy drinks as calculated from chemometrics prediction were comparable to the results determined using a thin-layer chromatography–densitometry method developed from a previous study.

Keywords: caffeine, multivariate calibration, nicotinamide, pyridoxine, spectroscopy

INTRODUCTION

Humans need energy to carry out activities of daily living (Burger, Delong, & Hamilton, 2011). Energy can be obtained from several sources such as food consumed daily and from supplements (Maughan, Depiesse, & Geyer, 2007). Food supplements can be defined as products intended to supplement the diet and that contain one or more of the following food ingredients such as vitamins, minerals, herbs or botanicals, and amino acids; dietary substances to supplement the diet by increasing total food intake; concentrates, metabolites, constituents, extracts or combinations of these ingredients (Buell, Frank, Ransone et al., 2013). Energy drinks, a popular type of food supplement, increase in sales as they can be consumed by people of various age ranges in more than 140 countries (Seifert, Schaechter, Hershorin et al., 2011). Energy drinks contain caffeine combined with taurine, glucuronolactone, guarana and B vitamins to form a drink formula that manufacturers often name as the 'energy blend' (Higgins, Tuttle, & Higgins, 2010).

Caffeine is one of the main components in energy drinks (Attipoe, Leggit, & Deuster, 2016). A psychoactive compound, caffeine has biological effects such as enhancing endurance, increasing strength and work duration as well as reducing drowsiness (Babu, Church, & Lewander, 2008). However, high caffeine consumption, such as by drinking excessive energy drinks, may cause serious health consequences such as seizure, stroke and sudden death (Broderick & Benjamin, 2004; Clauson, Shields, McQueen et al., 2008; (Seifert, Schaechter, Hershorin et al., 2011). To overcome the various health problems stemming from the overconsumption of caffeinated beverages, it is important to enforce regulation concerning labelling, and advertising or carrying out health campaigns on the implications of caffeine levels in dietary supplements (Reissig, Strain, & Griffiths, 2009). Further, it is important to determine the content of caffeine and water-soluble vitamins such as nicotinamide (vitamin

B3) and pyridoxine (vitamin B6) in energy drinks to control the caffeine content and to maintain the nutritional adequacy of energy drinks (Gliszczyńska-Świgło & Rybicka, 2015).

Analysis of caffeine, nicotinamide and pyridoxine has been performed using several chromatographic methods such as high-performance liquid chromatography (HPLC) (Gliszczyńska-Świgło & Rybicka, 2015), thin-layer chromatography (TLC)-densitometry (Riswanto, Endang Lukitaningsih, & Martono, 2015), surfactant-mediated matrix-assisted laser desorption/ionization (Grant & Helleur, 2008), and planar chromatography–electrospray ionization mass spectrometry (Aranda & Morlock, 2006). However, chromatographic methods involve a separation process, are time-consuming, relatively expensive, and require special expertise in the field of separation science. Hence, it is important to develop a fast, simple and low-cost method for analysing caffeine, nicotinamide and pyridoxine content simultaneously.

Ultraviolet (UV) spectroscopy combined with multivariate calibration techniques can be used to overcome the problem of analyte determination in the mixture matrix (Dzulfianto, Riswanto, & Rohman, 2017; Suhandy & Yulia, 2017). Here, we aimed to develop a fast, simple, applicable and economical method for determining the caffeine, nicotinamide and pyridoxine content in simulated energy drinks. The predicted values obtained from the predictive UV spectroscopy were compared with the actual values obtained according to the validated TLC-densitometry method described in a previous study (Riswanto, Endang Lukitaningsih, & Martono, 2015).

MATERIALS AND METHODS

Materials

The caffeine standard was obtained from PT Kalbe Farma (Jakarta, Indonesia). The pyridoxine and nicotinamide standards were obtained from PT Erela (Semarang, Indonesia). The solvents used in this study were methanol, ethyl acetate, 25% ammonia (Merck Millipore, Darmstadt, Germany) and redistilled water (PT Ikapharmindo Putramas, Jakarta, Indonesia). The TLC silica gel 60 F_{254} plates were purchased from Merck Millipore (Darmstadt, Germany). The simulated energy drink samples containing caffeine, nicotinamide and pyridoxine were formulated in the laboratory of the Faculty of Pharmacy, Sanata Dharma University, Indonesia.

Instruments and software: The instruments used in this study were a UV 1800 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 1 cm quartz cuvette (Hellma, Jena, Germany), UYA 2.3Y ultra-micro analytical balance with specification max, 2.1 g; min, 0.01 mg (RADWAG®, Radom, Poland) and a set of Socorex® (Ecublens, Switzerland) micropipettes. We also used an Automatic TLC Sampler Linomat 5 and TLC Scanner 3 system with 6.00 × 0.10 mm slit dimension and 20 mm/s scanning speed (CAMAG, Muttenz, Switzerland). The TLC scanner was controlled via the winCats 1.4.4.6337 Planar Chromatography Manager software platform (CAMAG, Muttenz, Switzerland). UV spectral acquisition was controlled and processed using UVProbe Software (Shimadzu, Japan). The UV spectral data were exported to Excel (Microsoft Inc, Washington, USA) and converted into .csv files. Multivariate calibrations and spectral preprocessing were carried out using the pls and prospectr packages, respectively, in RStudio 1.1.456.

Preparation of standard solutions: An accurate weight of 49.9 mg caffeine, 50.1 mg nicotinamide and 50.2 mg pyridoxine were transferred into three separate 50-ml volumetric flasks. The weighted standard for each volumetric flask were diluted in methanol to volume. These solutions were labelled as caffeine, nicotinamide and pyridoxine stock solution, respectively. Standard solutions for UV spectral scanning were prepared from the stock solutions for each compound. The stock solutions, i.e. 100 µl caffeine, 50 µl nicotinamide and 20 µl pyridoxine, were transferred into three separate 10-ml volumetric flasks, followed by methanol dilution to volume. A standard solution mixture was prepared by transferring 200, 100 and 40 µl caffeine, nicotinamide and pyridoxine stock solution, respectively, into a 10 ml volumetric flask, followed by methanol dilution to volume. Each solution was scanned using the UV spectrophotometer at 200–350 nm to depict the UV spectral profile for each compound as well as the mixture solution.

Caffeine, nicotinamide and pyridoxine standard solutions were also prepared for TLC-densitometry. A caffeine standard solution set was prepared by transferring 18, 24, 30, 36, 42 and 48 µL caffeine standard stock solution into a 5-ml volumetric flask separated for each concentration, followed by dilution to volume with water. A nicotinamide standard solution set was prepared by transferring 7.5, 12.5, 17.5, 22.5, 27.5 and 32.5 µL nicotinamide standard stock solution into a 5-ml volumetric flask separated for each concentration, followed by dilution to volume with water. A pyridoxine standard solution set was prepared by transferring 7, 10, 13, 16, 19 and

22 µL pyridoxine standard stock solution into a 5-ml volumetric flask separated for each concentration, followed by dilution to volume with water.

Preparation of calibration set and validation set solutions: A set of calibration solutions and validation solutions were prepared from the stock solutions to achieve 35 standard solution mixtures (Table 1). The calibration data set (25 composition variations) and the validation data set (10 composition variations) were composed randomly using Excel (Microsoft Inc.). Each mixture was scanned using the UV spectrophotometer at 200–350 nm. The absorbance for each mixture was recorded at intervals of 2 nm. The obtained absorbance data for each wavelength point were used for generating both the calibration and validation models.

Sample preparation: Samples of simulated energy drinks containing caffeine, nicotinamide and pyridoxine were homogenized. The samples (750 μl) were transferred into 10 ml volumetric flasks, followed by methanol dilution to volume. The prepared samples were analysed using both spectroscopy for generating the predictive models of multivariate calibration, and TLC-densitometry for determining the actual values for each compound in the samples.

TLC-densitometry: We used TLC-densitometry because it has been successfully validated previously (Riswanto, Endang Lukitaningsih, & Martono, 2015). All standards and samples were applied in the TLC plates used in the Automatic TLC Sampler Linomat 5 (CAMAG, Muttenz, Switzerland) with 3.0-mm band length, 9-mm track distance, 50 nL/s band velocity, and the first-application x-axis and y-axis were 10.0 and 15.0 mm, respectively. Before separation, the chamber was saturated with mobile-phase methanol–ethyl acetate–25% ammonia (13:77:10, v/v/v) for 30 min. Chromatographic separation was performed in a 20 × 20 cm flat bottom chromatographic chamber with a migration distance of 7.5 cm. After development, the plate was dried in a stream of warm air for 2 min, followed by detection using TLC Scanner 3 (CAMAG, Muttenz, Switzerland). In absorption mode, caffeine, nicotinamide and pyridoxine were measured at UV 274, 263 and 293 nm, respectively.

Spectroscopic analysis and multivariate calibration

The absorbance values of each wavelength point determined from the calibration and validation data sets were statistically analysed. Spectral preprocessing was performed with the prospectr package in RStudio. We generated original, first derivative, second derivative, standard normal variate (SNV) and Savitzky-Golay (SG) smoothing spectra with a window width of 11 points and polynomial order 3. Chemometrics data processing was performed using the pls package. We used two multivariate calibration techniques, namely principle component regression (PCR) and partial least squares (PLS) to generate an appropriate predictive model for each compound. The statistical parameters used for evaluating the multivariate calibrations performance were the coefficient of determination for calibration (R_{cal}^2), cross-validation (R_{CV}^2), validation (R_{val}^2), root mean square error of calibration (RMSEC), RMSE of cross-validation (RMSECV) and RMSE of prediction (RMSEP). The selected calibration model for each compound was analysed further to form a predictive equation generated from the actual versus predicted values.

Determination of caffeine, nicotinamide and pyridoxine

The prepared sample solutions were analysed using both spectroscopy and TLC-densitometry. The caffeine, nicotinamide and pyridoxine content determined using TLC-densitometry was stated as the actual value for generating predictive models. The sample solutions were also scanned using a spectrophotometer in the 200–350 nm range with an interval of 2 nm. Absorbance data obtained from each wavelength point were analysed statistically and plotted in the validation predictive plot and compared to the actual values from the TLC-densitometry. Sample determination was replicated five times. The error prediction of each compound in the mixture was calculated as the relative error of prediction (REP) using the Equation 1 (Hemmateenejad, Akhond, & Samari, 2007).

$$\mathrm{REP}\ (\%) - 100\ \mathrm{x} \sqrt{\frac{\sum_{j-1}^{N} (\hat{C}j - Cj)^2}{\sum_{j-1}^{N} (Cj)^2}} \quad \text{...Equation 1}$$

where, N is the total number of samples; $\hat{C}_i$ represents the predicted concentration for the j^{th} sample, and C_i the actual concentration from TLC-densitometry.

RESULTS AND DISCUSSION

TLC-densitometry analysis of caffeine, nicotinamide and pyridoxine

TLC-densitometry was chosen as the reference method because it has been validated and presented previously (Riswanto, Endang Lukitaningsih, & Martono, 2015). Figure 1 depicts the representative chromatogram profile of the sample. The assay's selectivity, accuracy, precision, linearity, limit of detection and limit of

quantitation can also be used for quantitatively determining caffeine, nicotinamide and pyridoxine in the energy drinks sample. Hence, it was possible to use the chromatographic quantification results as the actual value for further multivariate calibration and regression modelling (Martono, Riyanto, Martono et al., 2016). For quantitative determination, the multiple point calibration method was generated from each compound to calculate the actual caffeine, nicotinamide and pyridoxine content. The equations for caffeine, nicotinamide and pyridoxine were $y = 22.306x + 776.56$ ($R^2 = 0.996$), $y = 10.131x + 350.33$ ($R^2 = 0.998$) and $y = 25.108x - 314.06$ ($R^2 = 0.995$), respectively.

Spectroscopic analysis and multivariate calibration

Spectroscopic analysis was initially performed with UV spectral profile observation. Single standards of 9.98 μg/ml caffeine, 5.01 μg/ml nicotinamide and 2.01 μg/ml pyridoxine were scanned separately at the UV range of 200–350 nm. The mixed standard solution containing 19.96 μg/ml caffeine, 10.02 μg/ml nicotinamide and 4.02 μg/ml pyridoxine was also scanned at the same wavelength range. Both the single and mixed standards were scanned at 2-nm intervals and displayed in overlaying mode to support visual observation of the spectra. Calibration set solutions were also scanned at the same wavelength range and interval. Figure 2 shows the UV spectra profiles of caffeine, nicotinamide, pyridoxine and the mixed standards, and the scanning results of the calibration set solutions. The overlaid UV spectra were examined and showed that it was difficult to determine the caffeine, nicotinamide and pyridoxine content in the mixture with conventional spectroscopy techniques (El-Ghobashy & Abo-Talib, 2010). Separation techniques such as chromatography enabled quantitative analysis of single components in the mixture matrix (20, 21). On the other hand, the chromatographic techniques were time-consuming and costly (Dwiastuti, Marchaban, Istyastono et al., 2018; Yuliani, Istyastono, & Riswanto, 2016) compared to the spectroscopic techniques ((Dzulfianto, Riswanto, & Rohman, 2017; Glavanović, Glavanović, & Tomišić, 2016). Using multivariate calibrations as chemometrics techniques enabled analysis of the caffeine, nicotinamide and pyridoxine content simultaneously in the mixture using spectroscopy. A calibration standard solution set with 25 composition variations were scanned with 2-nm intervals and yielded the absorbance value for each wavelength point. All scanning results were recorded as raw data for generating multivariate calibration models in the further analysis.

Here, we generated original, first derivative, second derivative, SNV, and SG smoothing spectra with a window width of 11 points and polynomial order 3 using the R package prospectr (Stevens & Ramirez-López, 2014). Prospectr was useful for preprocessing spectral data and is commonly applied in spectroscopy analysis. Here, the goal of spectral preprocessing was to improve the subsequent bilinear calibration model (Rinnan, Berg, & Engelsen, 2009). The multivariate calibration models we developed were PCR and PLS. All models were generated using RStudio with the pls package (R Development Core Team 3.5.1., 2018; Mevik & Wehrens, 2015). Basically, PCR reduces the number of predictor variables by using their first few principal components rather than the original variables, while PLS regression utilizes the linear combinations of the predictor variables rather than the original variables (Miller & Miller, 2010). Table 2, Table 3, and Table 4 shows the performance of PCR and PLS for predicting the caffeine, nicotinamide and pyridoxine content statistical parameters such as R^2 (R_{cal}^2, R_{CV}^2, R_{val}^2) and RMSE (RMSEC, RMSECV, RMSEP) were considered to select the calibration model for each compound. Multivariate calibration models with high R^2 scores and the lowest RMSE (root mean square error) value were selected (Irnawati, Riyanto, Martono et al., 2019). The multivariate calibration models for caffeine, nicotinamide and pyridoxine were PLS (original/normal spectra), PCR (original/normal spectra), and PCR (SG smoothing spectra), respectively. To avoid over-optimistic prediction, the number of components for each compound were selected according to the lowest RMSECV value. The optimal number of components each for caffeine, nicotinamide and pyridoxine using the selected calibration models was 5, 10 and 13, respectively (Figure 3). Figure 4 depicts the prediction plots for caffeine, nicotinamide and pyridoxine of the selected models generated from the calibration and validation data sets. The equations used for correlating between the actual and predicted values for caffeine, nicotinamide and pyridoxine were $y = 1.068x - 0.889$ ($R^2 = 0.988$), $y = 1.237x - 1.923$ ($R^2 = 0.975$) and $y = 1.150x - 0.722$ ($R^2 = 0.977$), respectively. Figure 5 shows the regression coefficient plots for caffeine, nicotinamide and pyridoxine of the selected models. The plots were useful by plotting the point estimates of coefficients and the confidence intervals of different variables (Jann, 2014). Several peaks and valleys at certain wavelengths were indicated as important and considered for

analysis of caffeine, nicotinamide and pyridoxine (Suhandy & Yulia, 2017). We noted that all important wavelengths were in the UV region. Hence, it was clear that UV spectroscopy was appropriate for use in this study.

Determination of caffeine, nicotinamide and pyridoxine

The selected models were further used for analysing simulated energy drinks containing caffeine, nicotinamide and pyridoxine to obtain the calculated value for each determination. The calculated values were compared to the actual value obtained from the TLC-densitometry analysis. Statistical evaluations were performed by assessing the percentage of relative standard deviation (RSD) and relative error of prediction (REP). The RSD expresses the precision of the mean for the results obtained, while the REP expresses the error prediction of each compound. Table 5 shows, the RSD and REP of caffeine and nicotinamide determination reported low values and indicated the precision with a lower error of predictive ability. In pyridoxine determination, the RSD values were <10%, indicating the precision of the data, while the REP value was >10%. However, pyridoxine was found in low concentrations. The sensitivity of the method should be observed further, as it has been reported that the concentration can affect the predictive ability of multivariate calibration (Sim & Jeffrey Kimura, 2019).

CONCLUSION

We successfully developed UV spectroscopy combined with multivariate calibrations as alternative techniques for simultaneous determination of caffeine, nicotinamide and pyridoxine content in simulated energy drinks without requiring separation. The multivariate calibration models for caffeine, nicotinamide and pyridoxine were PLS with original/normal spectra, PCR with original/normal spectra, and PCR with SG smoothing spectra, respectively. This method is simple, rapid, effective, low-cost and comparable to the validated TLC-densitometry method. However, it is important to develop multivariate calibration analysis using actual commercial energy drink samples.

ACKNOWLEDGEMENT

The authors are grateful to PT Kalbe Farma and PT Erela for supporting working standards, Faculty of Pharmacy Sanata Dharma University for providing facilities, and Institute for Research and Community Services Sanata Dharma University for financially funding by The Young Lecturer Research Grant of 2020 (005/Penel./LPPM-USD/I/2020) awarded to Michael Raharja Gani.

REFERENCES

1. Aranda, M., & Morlock, G. (2006). Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry. Journal of Chromatography A, 1131(1–2), 253–260. https://doi.org/10.1016/j.chroma.2006.07.018
2. Attipoe, S., Leggit, J., & Deuster, P. A. (2016). Caffeine content in popular energy drinks and energy shots. Military Medicine, 181(9), 1016–1020. https://doi.org/10.7205/MILMED-D-15-00459
3. Babu, K. M., Church, R. J., & Lewander, W. (2008). Energy Drinks: The New Eye-Opener For Adolescents. Clinical Pediatric Emergency Medicine, 9(1), 35–42. https://doi.org/10.1016/j.cpem.2007.12.002
4. Broderick, P., & Benjamin, A. B. (2004). Caffeine and psychiatric symptoms: a review. The Journal of the Oklahoma State Medical Association, 97(12), 538–542.
5. Buell, J. L., Franks, R., Ransone, J., Powers, M. E., Laquale, K. M., & Carlson-Phillips, A. (2013). National athletic trainers' association position statement: Evaluation of dietary supplements for performance nutrition. Journal of Athletic Training, 48(1), 124–136. https://doi.org/10.4085/1062-6050-48.1.16
6. Burger, O., Delong, J. P., & Hamilton, M. J. (2011). Industrial energy use and the human life history. Scientific Reports, 1. https://doi.org/10.1038/srep00056
7. Clauson, K. A., Shields, K. M., McQueen, C. E., & Persad, N. (2008). Safety issues associated with commercially available energy drinks. Journal of the American Pharmacists Association. 48(3), 55-63. https://doi.org/10.1331/JAPhA.2008.07055
8. Dwiastuti, R., Marchaban, Istyastono, E. P., & Riswanto, F. D. O. (2018). Analytical method validation and determination of free drug content of 4-n-butylresorcinol in complex lipid nanoparticles using RP-HPLC method. Indonesian Journal of Chemistry, 18(3), 496–502. https://doi.org/10.22146/ijc.28919
9. Dzulfianto, A., Riswanto, F. D. O., & Rohman, A. (2017). The employment of UV-spectroscopy combined with multivariate calibration for analysis of paracetamol, propyphenazone and caffeine. Indonesian Journal of Pharmacy, 28(4), 191–197. https://doi.org/10.14499/indonesianjpharm28iss4pp191
10. El-Ghobashy, M. R., & Abo-Talib, N. F. (2010). Spectrophotometric methods for the simultaneous determination of binary mixture of

metronidazole and diloxanide furoate without prior separation. Journal of Advanced Research, 1(4), 323–329. https://doi.org/10.1016/j.jare.2010.06.001

11. Glavanović, S., Glavanović, M., & Tomišić, V. (2016). Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 157, 258–264. https://doi.org/10.1016/j.saa.2015.12.020
12. Gliszczyńska-Świgło, A., & Rybicka, I. (2015). Simultaneous Determination of Caffeine and Water-Soluble Vitamins in Energy Drinks by HPLC with Photodiode Array and Fluorescence Detection. Food Analytical Methods, 8(1), 139–146. https://doi.org/10.1007/s12161-014-9880-0
13. Grant, D. C., & Helleur, R. J. (2008). Simultaneous analysis of vitamins and caffeine in energy drinks by surfactant-mediated matrix-assisted laser desorption/ionization. Analytical and Bioanalytical Chemistry, 391(8), 2811–2818. https://doi.org/10.1007/s00216-008-2207-5
14. Hemmateenejad, B., Akhond, M., & Samari, F. (2007). A comparative study between PCR and PLS in simultaneous spectrophotometric determination of diphenylamine, aniline, and phenol: Effect of wavelength selection. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 67(3–4), 958–965. https://doi.org/10.1016/j.saa.2006.09.014
15. Higgins, J. P., Tuttle, T. D., & Higgins, C. L. (2010). Energy beverages: Content and safety. Mayo Clinic Proceedings. https://doi.org/10.4065/mcp.2010.0381
16. Irnawati, Riyanto, S., Martono, S., & Rohman, A. (2019). Analysis of palm oil as oil adulterant in olive and pumpkin seed oils in ternary mixture systems using ftir spectroscopy and chemometrics. International Journal of Applied Pharmaceutics, 11(5), 210–215. https://doi.org/10.22159/ijap.2019v11i5.34274
17. Jann, B. (2014). Plotting regression coefficients and other estimates. Stata Journal, 14(4), 708–737. https://doi.org/10.1177/1536867x1401400402
18. Martono, Y., Riyanto, S., Martono, S., & Rohman, A. (2016). Determination of stevioside and rebaudioside a from simulated stevia beverages using FTIR spectroscopy in combination with multivariate calibration. Research Journal of Medicinal Plant, 10(5), 349–355. https://doi.org/10.3923/rjmp.2016.349.355
19. Maughan, R. J., Depiesse, F., & Geyer, H. (2007). The use of dietary supplements by athletes. The use of dietary supplements by athletes. Journal of sports sciences, 25 Suppl 1, S103–S113. https://doi.org/10.1080/02640410701607395
20. Mevik, B.-H., & Wehrens, R. (2015). Introduction to the pls Package. Help Section of The "pls" package of RStudio Software, (Section 7), 1–23.
21. Miller, J. N., & Miller, J. C. (2010). Statistics and Chemometrics for Analytical Chemistry. 6th ed. Harlow, England: Pearson/Prentice Hall.
22. R Development Core Team 3.5.1. (2018). A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (Vol. 2). Retrieved from http://www.r-project.org.
23. Reissig, C. J., Strain, E. C., & Griffiths, R. R. (2009). Caffeinated energy drinks--a growing problem. Drug and alcohol dependence, 99(1-3), 1–10. https://doi.org/10.1016/j.drugalcdep.2008.08.001
24. Rinnan, Å., Berg, F. van den, & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. TrAC - Trends in Analytical Chemistry, 28(10), 1201-1222. https://doi.org/10.1016/j.trac.2009.07.007
25. Riswanto, F. D. O., Endang Lukitaningsih, R. R., & Martono, S. (2015). Analytical method validation and determination of pyridoxine, nicotinamide, and caffeine in energy drinks using thin layer chromatography-densitometry. Indonesian Journal of Chemistry, 15(1), 9–15. https://doi.org/10.22146/ijc.21217
26. Seifert, S. M., Schaechter, J. L., Hershorin, E. R., & Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. Pediatrics, 127(3), 511–528. https://doi.org/10.1542/peds.2009-3592
27. Sim, S. F., & Jeffrey Kimura, A. L. (2019). Partial least squares (pls) integrated fourier transform infrared (ftir) approach for prediction of moisture in transformer oil and lubricating oil. Journal of Spectroscopy, 2019. https://doi.org/10.1155/2019/5916506
28. Stevens, A., & Ramirez-López, L. (2014). Miscellaneous functions for processing and sample selection of vis-NIR diffuse reflectance data. CRAN "prospectr" R. Retrieved from https://github.com/antoinestevens/prospectr
29. Suhandy, D., & Yulia, M. (2017). The Use of Partial Least Square Regression and Spectral Data in UV-Visible Region for Quantification of Adulteration in Indonesian Palm Civet Coffee. International Journal of Food Science, 2017, 6274178. https://doi.org/10.1155/2017/6274178
30. Yuliani, S. H., Istyastono, E. P., & Riswanto, F. D. O. (2016). The cytotoxic activity on T47D breast cancer cell of genistein-standardized ethanolic extract of tempeh - A fermented product of soybean (Glycine max). Oriental Journal of Chemistry, 32(3), 1619–1624. https://doi.org/10.13005/ojc/320338.

TABLES AND FIGURES

Table 1: Calibration and validation data set information for model selection and statistical analysis for determining caffeine, nicotinamide and pyridoxine content

Item	Data set	
	Calibration	Validation
Number of mixture standards	25	10
Caffeine concentration (μg/ml)		
Mean	11.445	12.395
Range	2.794–29.84	3.693–26.746
Nicotinamide concentration (μg/ml)		
Mean	8.405	8.667
Range	1.603–14.228	0.20–14.93
Pyridoxine concentration (μg/ml)		
Mean	4.434	4.468
Range	0.904–9.839	1.004–8.735
Multivariate calibration models	PCR PLS	PCR PLS
Evaluated parameters for model selection	R_{cal}^2 RMSEC RMSECV* and R_{CV}^2	R_{val}^2 RMSEP

* Cross-validation was performed using the leave-one-out technique.

Table 2: The performance of PCR and PLS for predicting caffeine content

Analyte	Multivariate calibration	Spectrum	Number of components	R_{cal}^2	RMSEC	R_{CV}^2	RMSECV	R_{val}^2	RMSEP
Caffeine	PCR	Original	11	0.996	0.466	0.986	0.796	0.954	1.832
		First derivative	4	0.986	0.831	0.977	1.015	0.955	1.823
		Second derivative	10	0.992	0.616	0.973	1.115	0.958	1.751
		SNV	16	0.999	0.244	0.987	0.755	0.939	2.108
		SG	10	0.996	0.423	0.987	0.783	0.974	1.385
	PLS	**Original**	**5**	**0.993**	**0.591**	**0.984**	**0.839**	**0.988**	**0.952**
		First derivative	12	0.998	0.342	0.980	0.946	0.962	1.676
		Second derivative	8	0.993	0.595	0.975	1.075	0.966	1.571
		SNV	15	0.999	0.229	0.987	0.770	0.941	2.081
		SG	8	0.996	0.442	0.987	0.754	0.971	1.467

Note: The selected calibration models for each compound are marked in bold.

Table 3: The performance of PCR and PLS for predicting nicotinamide content

Analyte	Multivariate calibration	Spectrum	Number of components	R_{cal}^2	RMSEC	R_{CV}^2	RMSECV	R_{val}^2	RMSEP
Nicotinamide	PCR	**Original**	**10**	**0.995**	**0.335**	**0.981**	**0.613**	**0.975**	**0.878**
		First derivative	16	0.996	0.286	0.972	0.748	0.925	1.502
		Second derivative	13	0.984	0.586	0.940	1.102	0.943	1.312
		SNV	4	0.942	1.13	0.893	1.473	0.856	2.083
		SG	7	0.993	0.395	0.981	0.613	0.968	0.974
	PLS	Original	7	0.994	0.370	0.978	0.668	0.971	0.937
		First derivative	13	0.996	0.298	0.965	0.838	0.912	1.634
		Second derivative	7	0.975	0.748	0.940	1.100	0.902	1.717
		SNV	4	0.948	1.072	0.889	1.499	0.859	2.062
		SG	7	0.994	0.377	0.984	0.568	0.971	0.929

Note: The selected calibration models for each compound are marked in bold.

Table 4: The performance of PCR and PLS for predicting pyridoxine content

Analyte	Multivariate calibration	Spectrum	Number of components	R_{cal}^2	RMSEC	R_{CV}^2	RMSECV	R_{val}^2	RMSEP
Pyridoxine	PCR	Original	15	0.996	0.207	0.965	0.565	0.946	0.785

		First derivative	16	0.998	0.143	0.980	0.431	0.932	0.872
		Second derivative	21	0.997	0.180	0.909	0.918	0.903	1.045
		SNV	6	0.937	0.7901	0.862	1.128	0.784	1.561
		SG	**13**	**0.995**	**0.222**	**0.965**	**0.565**	**0.977**	**0.507**
	PLS	Original	9	0.992	0.280	0.963	0.586	0.862	1.247
		First derivative	14	0.998	0.133	0.975	0.479	0.923	0.930
		Second derivative	12	0.994	0.245	0.893	0.995	0.878	1.173
		SNV	3	0.895	1.027	0.844	1.198	0.873	1.197
		SG	9	0.991	0.297	0.964	0.579	0.975	0.523

Note: The selected calibration models for each compound are marked in bold.

Table 5: Results of caffeine, nicotinamide and pyridoxine content determination in simulated energy drink samples

No.	Caffeine		Nicotinamide		Pyridoxine	
	Actual	Calculated	Actual	Calculated	Actual	Calculated
1	332.160	333.347	143.600	135.490	44.747	40.001
2	340.627	329.275	143.507	141.417	46.160	41.127
3	333.867	334.584	138.053	142.624	44.013	44.356
4	333.120	333.089	135.973	131.279	43.893	35.833
5	335.907	332.383	141.827	133.690	43.347	43.565
Mean	335.136	332.535	140.592	136.900	44.432	40.976
RSD (%)	1.004	0.598	2.434	3.598	2.447	8.235
REP (%)		1.597		4.258		10.696

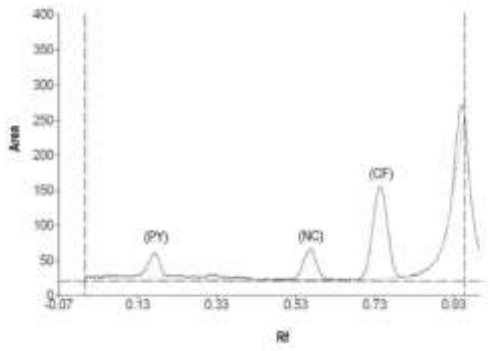

Fig.1: Representative chromatogram of simulated energy drink containing caffeine, nicotinamide and pyridoxine. Chromatographic conditions: mobile phase, methanol–ethyl acetate–25% ammonia (13:77:10 v/v/v); CF: caffeine; NC: nicotinamide; PY: pyridoxine. UV detection at 263 nm.

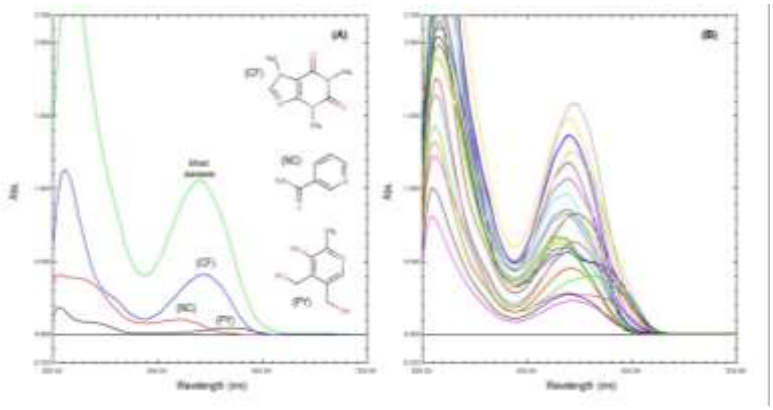

Fig.2: The UV spectra profiles of caffeine (CF), nicotinamide (NC), pyridoxine (PY) and mixed standards (A) and the scanning results of the calibration set solutions (B).

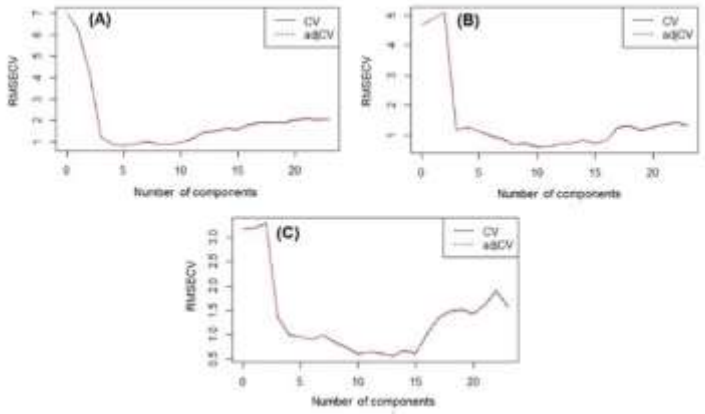

Fig.3: Component number selection for caffeine (A), nicotinamide (B) and pyridoxine (C) of the selected models.

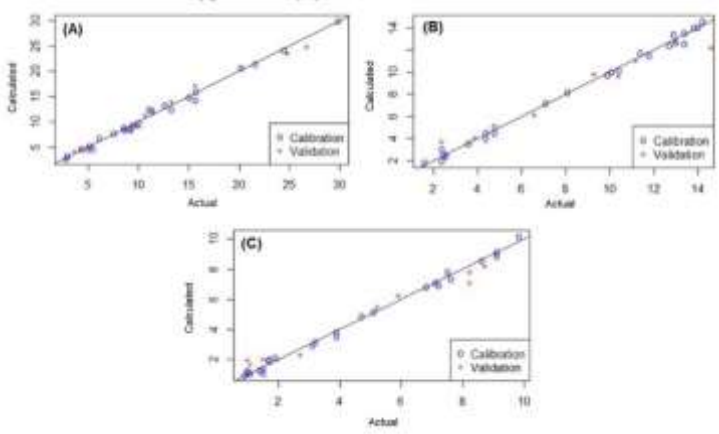

Fig.4: Prediction plots for caffeine (A), nicotinamide (B) and pyridoxine (C) of the selected models.

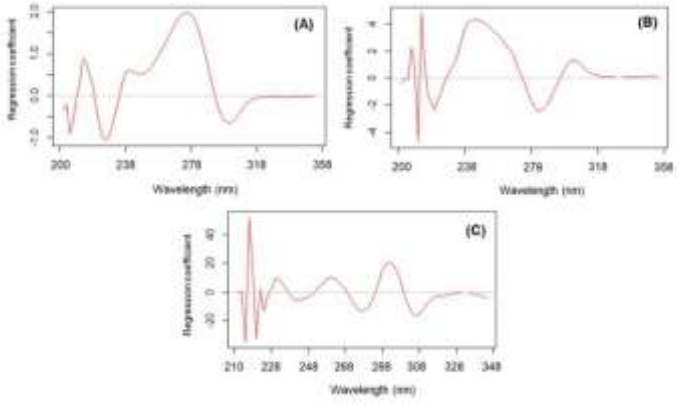

Fig.5: Regression coefficient plots for caffeine (A), nicotinamide (B) and pyridoxine (C) of the selected models.