Acta Pharm. Sci. Vol 60:(1), 2022
DOI: 10.23893/1307-2080.APS.6002

Application of Near-infrared Spectroscopy and Multivariate Methods for the Estimation of Isopropyl Alcohol Content in Hand Sanitizer Formulation

Sathish Dharani*[1], Tahir Khuroo[1], Sogra F. Barakh Ali[1]

1 Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, Texas A&M University, College Station, TX 77843, USA

ABSTRACT

To address the need of alcohol-based hand sanitizers during COVID-19, U.S. FDA has issued a guidance for the preparation of hand sanitizers that recommends 80% v/v ethanol or 75%v/v isopropyl alcohol (IPA) along with other ingredients. The aim of this study was to develop a new method to estimate IPA content in hand sanitizers by using Near-infrared (NIR) spectroscopy with a multivariate chemometric approach. Calibration samples containing 10-90% of IPA were used for model development. NIR data was mathematically pretreated with multiple scattering correction before development of partial least squares (PLSR) and principal component regressions (PCR) model. Both models showed good linearity over the selected range of IPA content with high R2 (>0.993), low root mean squared error (<2.163), minimum difference between standard errors between calibration and validation models (0.0009). The proposed NIR with multivariate methods provide rapid analysis of IPA content in the hand sanitizer.

Keywords- Isopropyl alcohol, disinfectant, near infrared, partial least square, calibration validation

INTRODUCTION

Hand sanitizer, also called hand rub or hand antiseptic, is applied to hands to protect from common pathogens when washing hands using soap is not an

*Corresponding author:
Sathish Dharani, Ph.D., 301 Reynolds Medical Building, College Station, Texas 77843-1114
Email: dharani@tamu.edu , Phone: 979-436-0608
ORCIDs:
Sathish Dharani- 0000-0002-4442-3780
Tahir Khuroo- 0000-0001-7683-5635
Sogra F. Barakh Ali- 0000-0002-9327-5737
(Received 4 Nov 2020, Accepted 15 Apr 2021)

available option [1, 2]. These products are made available in different forms such as foam, gel, or liquid, and majority of them are alcohol-based preparations. Though less effective, non-alcohol-based sanitizers are also available, and have triclosan or benzalkonium chloride as active ingredients [3, 4]. Centers for Disease Control and Prevention recommends use of alcohol-based hand sanitizers with greater than 60% ethanol or 70% isopropanol [5]. As a broad-spectrum bactericidal agent alcohol acts by either breaking proteins, splitting cells or interfering with a cell's metabolism [6-9]. The virucidal activity of alcohol is proportional to their concentration. Higher concentrations of ethanol (95%) generally have better virucidal activity than do lower concentrations, such as 60 to 80% and especially against naked viruses [10-13]. Likewise, the bactericidal activity of isopropanol begins at a concentration of 30% [14] and increases parallelly with increasing concentrations till 90% where after it shows a slight decline [15].

Due to the outbreak of novel coronavirus disease 2019 (COVID-19), hand sanitizers are flying off the shelves from grocery stores worldwide. Purchases of these products have skyrocketed in the U.S. from last week of February 2020, a period that saw the first American death from COVID-19. From March 2020 hand sanitizer market in the U.S. shot up by 470% with annual sales of more than $200 million compared to last year. In the early March 2020, an 8-ounce bottle sanitizer that would normally cost $2.50 was briefly on sale for $90 online [16]. An acute insufficiency was observed throughout the USA. To address shortage of sanitizers during this public emergency, the Food and Drug Administration (FDA) has issued a guidance for the preparation of alcohol or isopropyl alcohol (IPA) based hand sanitizers [17]. According to this guidance, the hand sanitizer product should contain 80%v/v ethanol or 75%v/v IPA in the formulation [17]. As most of the healthcare professionals and general public are relying on hand sanitizer as one of the preventing means, it is crucial to have a good quality control test to estimate the IPA or ethanol concentration in the final product. World Health Organization (WHO) recommends the use of alcohol based sanitizers with ethanol effective at 75%-85% (± 5%) and IPA at 77% (± 1%) and suggests alcoholmeter for quality control evaluation [18]. On the other hand, ethanol (60-95% v/v) and IPA (70-91.3% v/v) specifications are broad in FDA guidance document compared to WHO guidance. The agency recommended method for IPA and ethanol quantification are gas chromatography, alcoholmeter, hydrometer, or other equivalent method in terms of accuracy [17].

The burgeoning demand for sanitizers combined with the paucity of hydrometers makes it indispensable to develop an alternate analytical method to quantify alcohol content in hand sanitizer. Alternate analytical method could be ba-

sed on vibration spectroscopic methods. Near-infrared (NIR), a convenient and rapid vibration spectroscopic method, is becoming vital pharmaceutical tool of choice for nondestructive analysis where practically no sample preparation is required over a traditional wet chemistry method [19-24]. Unlike chromatographic methods, NIR peaks are not sharp due to higher order overtones and combination bands. Furthermore, the spectra may also be interfered by excipients present in the formulations. This results in a complex spectrum with overlapping and multiple bands of varying intensity/height, which required multivariate methods for quantitative estimation. Generally used multivariate analytical tools are principal component analysis (PCA) and projection to latent structures or partial least squares (PLS) [25]. The objective of this work is to combine NIR method with multivariate tools to build and validate chemometric models for quantification of IPA in FDA recommended hand sanitizer. This research work has not been reported in the literature to the best of our knowledge.

METHODOLOGY

Materials

IPA (USP grade 99%) was obtained from VWR International, LLC, Radnor, PA. Glycerol (USP/FCC grade,) and hydrogen peroxide (35% solution) were obtained from Fisher chemicals, Fair Lawn, NJ. Millipore water collected from Milli Q water system.

Manufacturing of hand sanitizer

Hand sanitizer was prepared as per FDA guidelines. It contained 75% v/v IPA, glycerin 1.45% v/v, hydrogen peroxide 0.125% v/v and water quantity sufficient to make 100% v/v. Briefly, glycerin and hydrogen peroxide were added to measured quantity of IPA. Volume was made up with water. The batch size was four liters, and twenty six batches were prepared. The batches were stored for 72 hours before complimentary distribution to various colleges of the university campus. Quarantine of 72 hours allow destruction of microbial spores, which may have formed during preparation steps [18].

Preparation of calibration samples

Calibration samples were prepared by the method described above. Glycerin and hydrogen peroxide content in the samples were identical to hand sanitizer formulations but contained varying percentage of IPA and water. IPA and water content varied 10-90%. 10 ml quantity was prepared for each sample in scintillation vials. All samples were characterized by NIR spectroscopy before developing multivariate models.

Near-Infrared spectroscopy

The NIR data of the samples was generated by using modular Nicolet™ iS™ 50 system (Thermo Fisher Scientific, Austin, TX). The instrument was equipped with a scanning grating monochromator and a diffuse reflectance apparatus (rapid content analyzer). NIR spectra ranging from 4000 to 10,000 cm^{-1} with a data resolution of 8 cm^{-1} and 100 scans were collected after conducting the diagnostic and reflectance tests. Prior to scanning, samples in a 20 ml borosilicate glass vial were mixed homogeneously by shaking, then placed on the sample window and centered with an iris. All samples were scanned in 6 replicates. Spectral acquisition was performed with OMNIC software, version 9.0.

Statistical analysis

Multivariate analysis of NIR data was performed using Unscrambler® X software (version 10.5; CAMO Software Inc., Woodbridge, NJ). Cross-validation approach was used to validate the models. The predictability of the models was further tested on independent samples. The performance of the chemometric models was evaluated in terms of correlation coefficient (R), determination coefficient (R^2), root-mean-squared error of calibration (RMSEC), root-mean-squared error of prediction (RMSEP), standard error of calibration (SEC), standard error of prediction (SEP) and bias.

RESULTS AND DISCUSSION

Spectral characterization

The NIR spectra of samples demonstrated broad bands due to vibrations of fundamental functional groups such as C-H, O-H, C-O and C-C [26, 27]. The spectra of IPA displayed characteristic bands at 8176, 8415, 8716, 9846 cm^{-1} and a trough at 7590 cm^{-1}. On the other hand, liquid water showed absorption band at 9330 cm^{-1}, and hydrogen peroxide exhibited absorption band at 9300 cm^{-1} with broader trough. Glycerol peak was characterized by a shoulder at 8269 cm^{-1} with troughs on both sides at 8874 and 7625 cm^{-1}. However, the IPA bands were not interfered by other components present in the formulation (**Figure 1**) making it an amenable method for its qualitative and quantitative estimation.

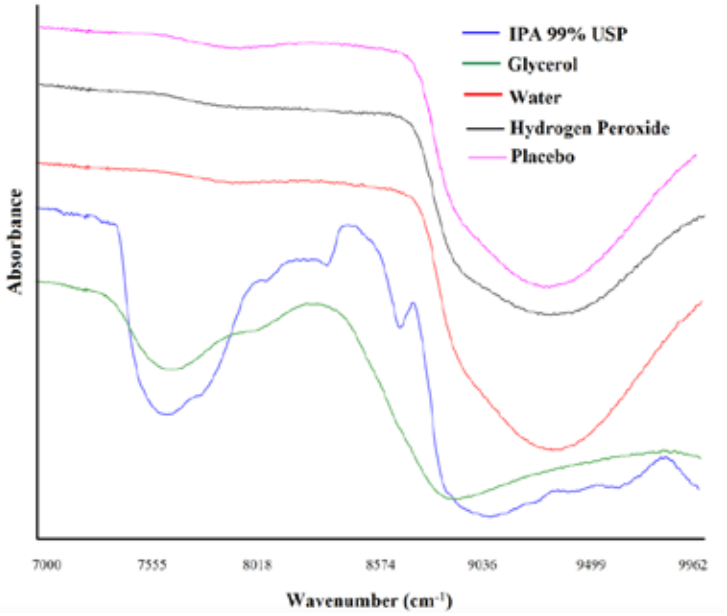

Figure 1. NIR spectra of IPA, water, glycerol, hydrogen peroxide and placebo sample

Chemometric analysis

Data processing

Truncated data of 7000-10000 cm^{-1} range was used for model development as it showed major bands of IPA. The data was mathematically pretreated with scatter correction methods such as extended multiple scattering correction (MSC) and standard normal variate (SNV), and spectral derivative method like Savitzky-Golay (SG), second derivative third-order polynomial with 9 smoothing points. The criterion for selection of mathematical method was based on values of R, R^2, standard errors (SEC and SEP), and root mean square errors (RMSEC and RMSEP) [23]. The pre-treatment methods are applied to individual spectra while mean centering and auto-scaling methods are applied to each individual variable of the samples [19, 20]. Single (MSC, SNV or SG), and combining two or more pretreatment methods (MSC-SNV, MSC-SG and MSC-SNV-SG) were explored to improve the quality of the data. Based on the values of statistical parameters (R, R^2, RMSEC, RMSEP, SEC and SEP) and spectral features, MSC method was selected for data treatment before models development (**Figure 2**).

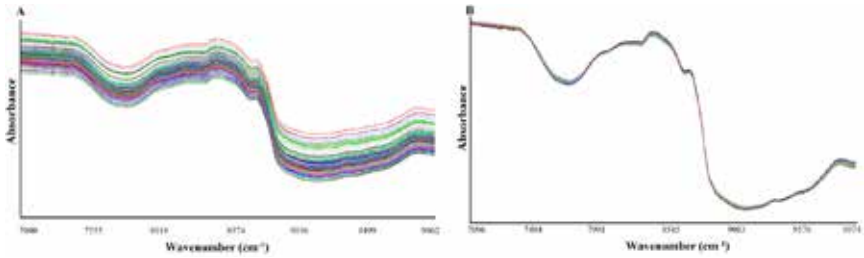

Figure 2. A) Truncated NIR, B) truncated NIR spectra pretreated with MSC

Regression models

PCA and PLS are data dimensionality techniques. PLS combine features of PCA and multiple linear regressions [28-30]. Both methods construct new predictor variables known as components PC (principle component) or least squares which is a linear combination of original predictor variables, but they construct those components in different ways. These components are also called as latent variables (LV). Principle component regression (PCR) generates components to describe the observed variability in the predictor variables, without considering the response variables. On the other hand, partial least squares regression (PLSR) does take the response variable into account, and hence frequently leads to models that are able to fit the response variable with fewer components. The model development starts with selection of number of LVs or PCs that would explain the variation in the data [31-33]. Number of LVs used in this model were optimized based on statistical parameters determination coefficient (R^2CV) and root-mean-square error of cross validation (RMSECV) [34, 35]. Two LVs were selected for model development with R^2_{CV} 0.994 and RMSEcv 2.053. These values were similar with three and four LVs, hence two LVs were used for model's development. The next steps in model development were detection and removal of the outliers from the dataset that has significant influence over the model prediction capability. Outlier detection was carried out using Hotelling's T^2 test at $p<0.01$, leverage and score plots. **Figure 3** showed Hotelling's plots of PCR and PLSR models. Hotelling's T^2 threshold limits at $p<0.01$ were 7.39 and 10.7 for first and second PC/LVs for both models, respectively. Hotelling's T^2 values of the samples were well below threshold limit (**Figure 3**).

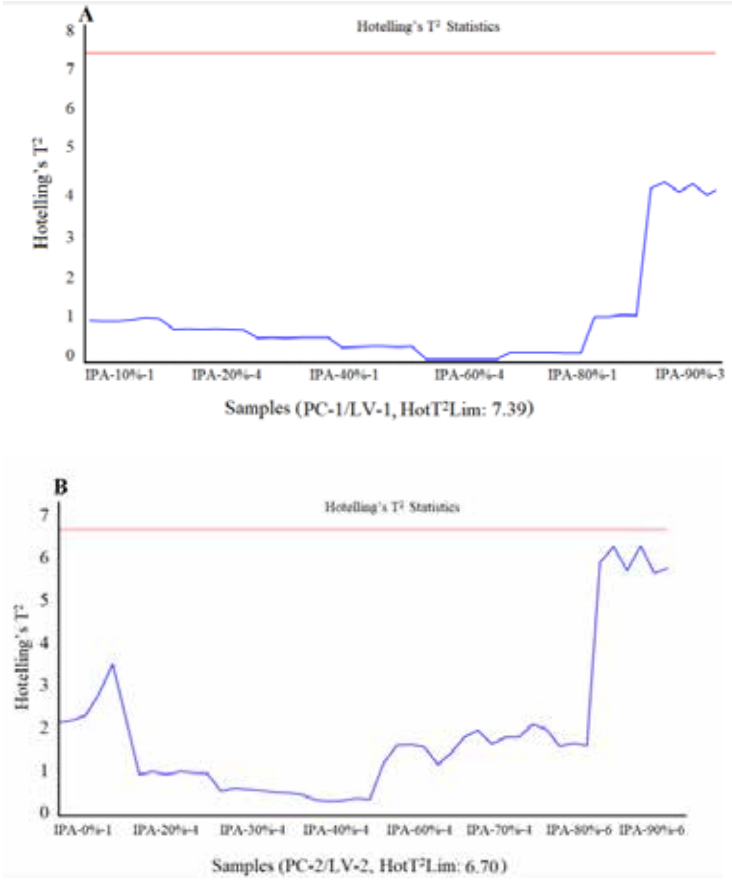

Figure 3. Hotelling T^2 curves for A) PC-1/LV-1 and B) PC-2/LV-2.

Leverage limit is defined by the formula 2A/I or 3A/I, where A is the number of LV and I is the number of samples. The leverage limit is used for quantifying the influence of sample on the model (**Figure 4**).

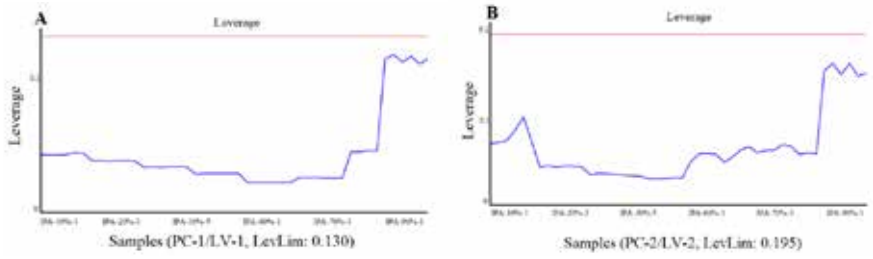

Figure 4. Leverage plots for A) PC-1/LV-1 and B) PC-2/LV-2.

The leverage limits were 0.130 and 0.195 for the first and second PCs/LVs for both models and samples were below the limits. Score plots of the samples between and first and second PC/LV are shown in Figure 5. Samples of identical concentration were clustered together that indicated samples belong to that particular group. Furthermore, score plots showed an increase in first PC/LV values with an increase in IPA concentration in the samples which indicated that first PC/LV was related to IPA in the samples. No such trend was observed in second PC/LV.

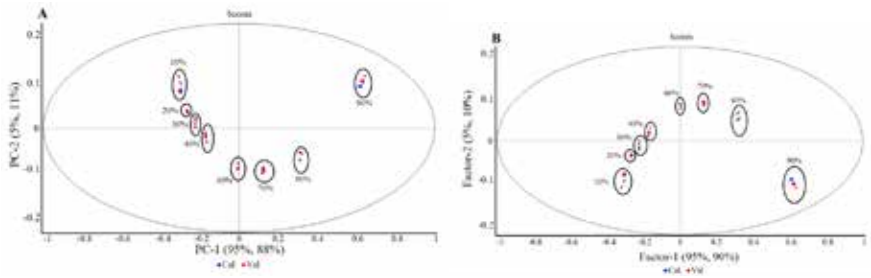

Figure 5. Score plots between A) PC-1 and PC-2 and B) LV-1 and LV-2.

Calibration and Internal validation

Initially, samples containing 0%-100% of IPA was used for model development with full cross validation wherein the same sample set of calibration models was used to validate the model. This is called internal validation. In this study, NIPALS algorithm was used for PLS and PCR regression models development [36]. Statistical parameters used to assess the calibration model are slope, offset, R, R^2, RMSEC, and SEC. Due to outlier detection in both PLSR and PCR models, samples containing 10-90% IPA was used in the final calibration set. The slope was close to 1 in both MSC treated PCR and PLS models, but the offset and RMSEC values were slightly higher in PCR model. The offset and RMSEC values of PCR and PLSR models were 0.306 and 0.276, and 2.162 and 2.052, respectively. However, the performance of calibration models was assessed by statistical parameters of the prediction model (**Figure 6**). The RMSEP and SEP values of PCR and PLSR models were 2.163 and 2.187, and 2.053 and 2.076, respectively. As the statistical parameters of both calibration and prediction were close to each other, the developed models would be considered a good fit models [19] (**Table 1**).

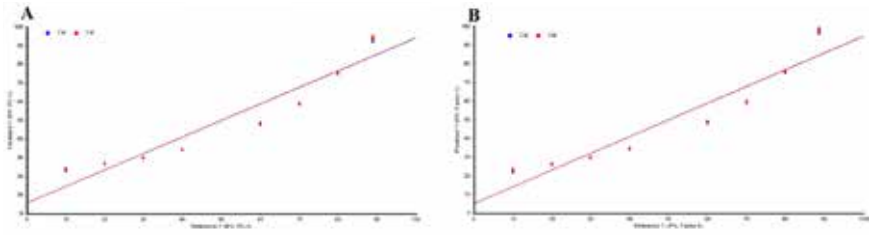

Figure 6. Calibration and validation plots for A) PCR and B) PLSR

FDA guidance document states that significant difference between SEC and SEP determines inadequacy of the model. The difference between SEP and SEC was less than 0.0009 for both PCR and PLSR models. The internal validation results showed good correlation between predicted and actual values for both PCR and PLSR models. Error in the model was estimated by residual values. Residual values between reference and model predicted values were low which indicated low error in the models.

Table 1. Statistical parameters of the model (pretreated with MSC)

Regression model	Model	Sample No.	Slope	Offset	Correlation	R^2	RMSEC (P)	SEC (P)	Bias
PCR	Calibration Validation	46 46	0.993 0.991	0.306 0.435	0.996 0.996	0.993 0.992	2.162 2.281	2.186 2.306	0 0.009
PLSR	Calibration Validation	46 46	0.994 0.992	0.276 0.394	0.997 0.996	0.994 0.993	2.052 2.174	2.075 2.198	0 0.008

RMSEC (P) – Root mean square error of calibration or prediction;

SEC (P) – Standard error of calibration or prediction.

Likewise, LV/PC in the loading plots of PLS/PCA regression model may provide the physical and chemical information of the samples by comparing spectra of individual components as well as formulations. The PLS1 showed all characteristic bands of IPA except the inversion of a trough at 9368 cm^{-1}. The PLS2 showed inverted peaks/valleys at 7536, 8369, 8709, 8805 and 9368 cm^{-1}. These bands were related to all the components of hand sanitizer formulations (**Figure 7**). Similarly, PC1 exhibited all the peaks of IPA, and PC2 exhibited peaks of all other components.

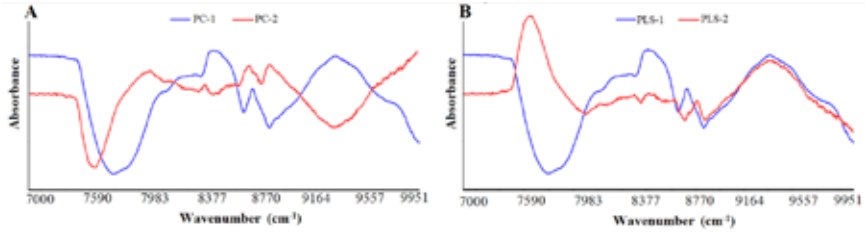

Figure 7. Loading plots of A) PCR and B) PLSR models

External validation of sanitizer batches

The PLS and PCR models were externally validated with independent samples of sanitizer batches which were not used in the model development. 10 mL of samples were collected in 20 mL scintillation vials from each batch. The NIR data of all batches was treated in the same way as was done on the samples used for model development. The data was plugged into the developed models after mathematical treatment. The amount of IPA predicted in the samples was close to the actual amount of 75%. The accuracy of the data was measured by residual values and the range was -3.74 to 1.78% for PCR, and -3.68 to 1.66% for PLSR. In general, residual value was lower in PLS models compared to PCR models. The minimum deviation was detected with batch #22 (1.81% for PCR and 1.73% for PLS) and maximum with batch #19 (3.67% for PCR and 3.49% for PLS) from the target values of IPA. All batches showed IPA content from 73.2±3.2% to 78.7±2.0% with PCR and 73.3±3.2% to 78.7±1.91% with PLS models (**Figure 8**). Batch #6 and #13 exhibited minimum and maximum IPA content for both models. Furthermore, predicted values of IPA by PCR and PLSR models overlapped for all the batches except batch #25 and #26. The PCR predicted values for batch #25 and #26 were 75.73±2.6 and 75.84±2.1%, respectively. Similarly, the PLS predicted values for batch #25 and 26 were 76.65±2.5 and 77.15±2, respectively. FDA guidelines dictates hand sanitizer formulation should contain 70-91.3% (v/v) IPA. Thus, the prepared formulation batches met IPA content criteria.

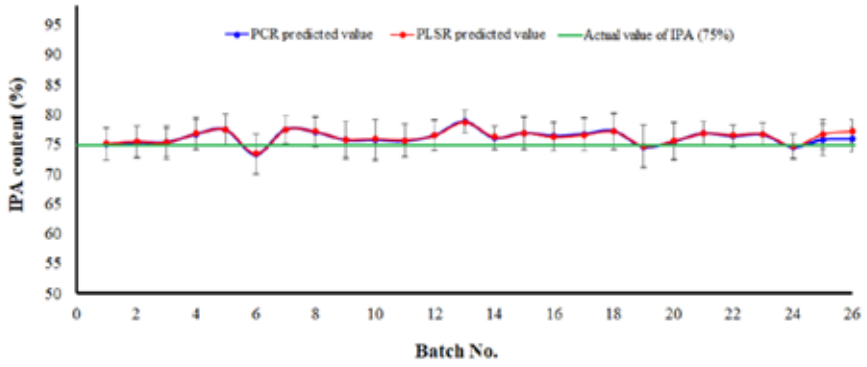

Figure 8. Comparison of PCR and PLSR predicted values with actual values of all batches.

The NIR spectroscopy methodology was developed for determination of IPA content in the hand sanitizer formulation. The peaks of IPA were distinct, not interfered by the other components of the formulation. A chemometric approach was combined with spectroscopy to achieve the goal of IPA prediction. Data was mathematically treated by various methods to improve its quality, detect and eliminate the outlier before development of PCR and PLSR models. MSC treated data set was used in PCR and PLS models development. The models showed high R^2 (>0.993), low RMSE (<2.163), and minimum difference between SEP and SEC (0.0009). The models were independently verified with unknown samples. The predicted values were in close concurrence with actual values with low residual (<3.76). The proposed analytical method is rapid and fast and, provide convenient way to measure IPA in the hand sanitizer. Thus NIR spectra can be used for qualitative and quantitative analysis in conjunction with multivariate method of IPA in the hand sanitizer formulation.

ACKNOWLEDGMENTS:

Authors are thankful to the Texas A&M Health Science Center for providing the facility to conduct this research.

CONFLICT OF INTEREST: Authors have no conflict of interest

AUTHOR CONTRIBUTIONS:

Sathish Dharani: Design of experiment, Drafting manuscript and statistical analysis

Tahir Khuroo: Preparation of sanitizer and Acquisition of data

Sogra F. Barakh Ali: Analysis of the data and statistical analysis

REFERENCES

1. Rai H, Knighton S, Zabarsky TF, Donskey CJ. Comparison of ethanol hand sanitizer versus moist towelette packets for mealtime patient hand hygiene. Am J Infect Control. 2017;45:1033-1034.

2. Babeluk R, Jutz S, Mertlitz S, Matiasek J, Klaus C. Hand Hygiene-Evaluation of Three Disinfectant Hand Sanitizers in a Community Setting. Plos One. 2014; 9(11):e111969. https://doi.org/10.1371/journal.pone.0111969.

3. *Gabriella B, Alexander KS*. Introduction to Cosmetic Formulation and Technology. *John Wiley & Sons. 2015*. https://www.wiley.com/en-us/Introduction+to+Cosmetic+Formulation+and+Technology-p-9781118763780.

4. https://www.ncbi.nlm.nih.gov/books/NBK144054/ (accessed 13 April 2020)

5. https://www.cdc.gov/coronavirus/2019-ncov/hcp/hand-hygiene.html. (accessed 15 April 2020)

6. Kampf G, Kramer A. Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs and Rubs. Clinical microbiology reviews. 2004; 17(4):863-893.

7. Hared R, Baik E, Gash S. Efficiency of antiseptics when acting on dried organisms. Br Med J. 1963;1: 496-500.

8. Hibbard SJ. Analyses Comparing the Antimicrobial Activity and Safety of Current Antiseptic Agents. J Infusion Nursing. 2005;28: 194-207.

9. Harrington C, Walker H. The germicidal action of alcohol. Boston Med Surg J. 1903;148: 548-552.

10. Eggers HJ. Experiments on antiviral activity of hand disinfectants. Some theoretical and practical considerations. Zentbl Bakteriol. 1990; 273: 36-51.

11. Steinmann J, Nehrkorn R, Meyer A. Two in vivo protocols for testing virucidal efficacy of handwashing and hand disinfection. Zentbl Hyg Umweltmed. 1995; 196: 425-436.

12. Best M, Springthorpe VS, Sattar SA. Feasibility of a combined carrier test for disinfectants: studies with a mixture of five types of microorganisms. Am J Infect Control. 1994; 22: 152-162.

13. Gershenfeld L. The sterility of alcohol. Am J Med Sci. 1938; 195: 358-361.

14. Powell UM. The antiseptic properties of isopropyl alcohol in relation to cold Sterilization. J Indiana State Med Assoc. 1945; 38: 303–304.

15. Tainter ML, Throndson AU, Beard RB. Chemical sterilization of instruments. J Am Dent Assoc. 1944; 31: 479–489.

16. https://www.cnbc.com/2020/03/27/coronavirus-the-history-of-hand-sanitizer-and-why-its-important.html. (Accessed 15 April 2020)

17. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-temporary-policy-preparation-certain-alcohol-based-hand-sanitizer-products-during. (Accessed 13 April 2020).

18. https://www.who.int/gpsc/5may/Guide_to_Local_Production.pdf (Accessed 15 April 2020).

19. Dharani S, Rahman Z, Barakh Ali SF, Afrooz H, Khan MA. Quantitative estimation of phenytoin sodium disproportionation in the formulations using vibration spectroscopies and multivariate methodologies. Int J Pharm. 2018; 539: 65-74.

20. Barakh Ali SF, Rahman Z, Dharani S, Afrooz H, Khan MA. Chemometric models for quan-

tification of carbamazepine and dihydrate forms in the formulation. J Pharm Sci. 2019; 108: 1211-1219.

21. Korang-Yeboah M, Akhtar S, Siddiqui A, Rahman Z, Khan MA. Application of NIR chemometric methods for quantification of the crystalline fraction of warfarin sodium in drug product. Drug Dev Ind Pharm. 2016; 42: 584-594.

22. Roggo Y, Chalus P, Maurer L, Lema-Martinez C, Edmond A, Jent N. A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. J Pharmaceut Biomed. 2007; 44: 683-700.

23. Rahman Z, Siddiqui A, Bykadi S, Khan MA. Near-infrared and fourier transform infrared chemometric methods for the quantification of crystalline tacrolimus from sustained-release amorphous solid dispersion. J Pharm Sci. 2014;103:2376–2385.

24. Zidan AS, Rahman Z, Sayeed V, Raw A, Yu L, Khan MA. Crystallinity evaluation of tacrolimus solid dispersions by chemometric analysis. Int J Pharm. 2012; 423: 341–350.

25. Dharani S, Barakh Ali SF, Afrooz H, Khan MA, Rahman Z. Univariate and multivariate models for determination of prasugrel base in the formulation of prasugrel hydrochloride using XRPD method. J Pharm Sci. 2019; 108: 3575-3581.

26. Westad F, Schmidt A, Kermit M. Incorporating chemical band-assignment in near infrared spectroscopy regression models. J Near Infrared Spectrosc. 2008; 16: 265–273.

27. Kutsyk AM, Ilchenko OO, Yuzvenko YM, Obukhovsky VV, Nikonova VV. Vibration spectroscopy of complex formation in aqueous solutions of isopropanol. Ukr J Phys. 2018; 63: 506-512.

28. Abdi H. Partial least squares regression and projection on latent structure regression (PLS Regression). Interdiscip Rev Comput Stat. 2010; 2: 97-106.

29. Godoy JL, Vega JR, Marchetti JL. Relationships between PCA and PLS-regression. Chemometrics and Intelligent Laboratory Systems. 2014; 130: 182–191.

30. Yan H, Ma Y, Xiong Z, Siesler HW, Qi L, Zhang G. Quantitative Analysis of Organic Liquid Three-Component Systems: Near-Infrared Transmission versus Raman Spectroscopy, Partial Least Squares versus Classical Least Squares Regression Evaluation and Volume versus Weight Percent Concentration Units. *Molecules*. 2019; *24:* 3564.

31. Amigo JM, Cruz J, Bautista M, Maspoch S, Coello J, Blanco M. Study of pharmaceutical samples by NIR chemical-image and multivariate analysis. Trends Anal Chem. 2008; 27: 696-713.

32. Porfire A, Filip C, Tomuta I. High-throughput NIR-chemometric methods for chemical and pharmaceutical characterization of sustained release tablets. J Pharm Biomed Anal. 2017; 138: 1-13.

33. Khorasani M, Amigo JM, Sun CC, Bertelsen P, Rantanen J. Near-infrared chemical imaging (NIR-CI) as a process monitoring solution for a production line of roll compaction and tableting. Eur J Pharm Biopharm. 2015; 93: 293-302.

34. Rahman Z, Zidan AS, Khan MA. Non-destructive methods of characterization of risperidone solid lipid nanoparticles. Eur J Pharm Biopharm. 2010; 76: 127-137.

35. Horikawa Y, Imai T, Takada R. et al. Chemometric analysis with near-infrared spectroscopy for chemically pretreated erianthus toward efficient bioethanol production. Appl Biochem Biotechnol. 2012; 166: 711-721.

36. Antonov L, Gergov G, Petrov V, Kubista M, Nygren J. UV–Vis spectroscopic and chemometric study on the aggregation of ionic dyes in water. Talanta.1999; 49: 99-106.