

# **Application of near-infrared spectroscopy and multivariate methods for the estimation of isopropyl alcohol content in hand sanitizer formulation**

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## **ABSTRACT**

To address the need of alcohol-based hand sanitizers during COVID-19, US 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 were 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 R<sup>2</sup> (>0.993), low rootbroad mean squared error (<2.163), minimum difference between standard errors between calibration and validation models (0.0009). The proposed NIR technique with multivariate approach provides 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 available option<sup>1,2</sup>. 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<sup>3,4</sup>. Centers for Disease Control and Prevention recommends use of alcohol-based hand sanitizers with greater than 60% ethanol or 70% isopropanol<sup>5</sup>. As a broad-spectrum bactericidal agent alcohol acts by either breaking proteins, splitting cells or interfering with a cell's metabolism<sup>6-9</sup>. 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<sup>10-13</sup>. Likewise, the bactericidal activity of isopropanol begins at a concentration of 30%<sup>14</sup> and increases parallelly with increasing concentrations till 90% where after it shows a slight decline<sup>15</sup>.

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 US 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 US 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<sup>16</sup>. 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<sup>17</sup>. According to this guidance, the hand sanitizer product should contain 80% v/v ethanol or 75% v/v IPA in the formulation<sup>17</sup>. 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% ( $\pm 5\%$ ) and IPA at 77% ( $\pm 1\%$ ) and suggests alcoholmeter for quality control evaluation<sup>18</sup>. 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<sup>17</sup>.

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 based 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<sup>19-24</sup>. 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)<sup>25</sup>. 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. Since the FDA has been issuing warnings about the sanitizers containing toxic methanol, we have taken initiation to develop this low cost NIR method to predict the quality of IPA based hand sanitizers. 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<sup>18</sup>.

### **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  $\text{cm}^{-1}$  with a data resolution of 8  $\text{cm}^{-1}$  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.

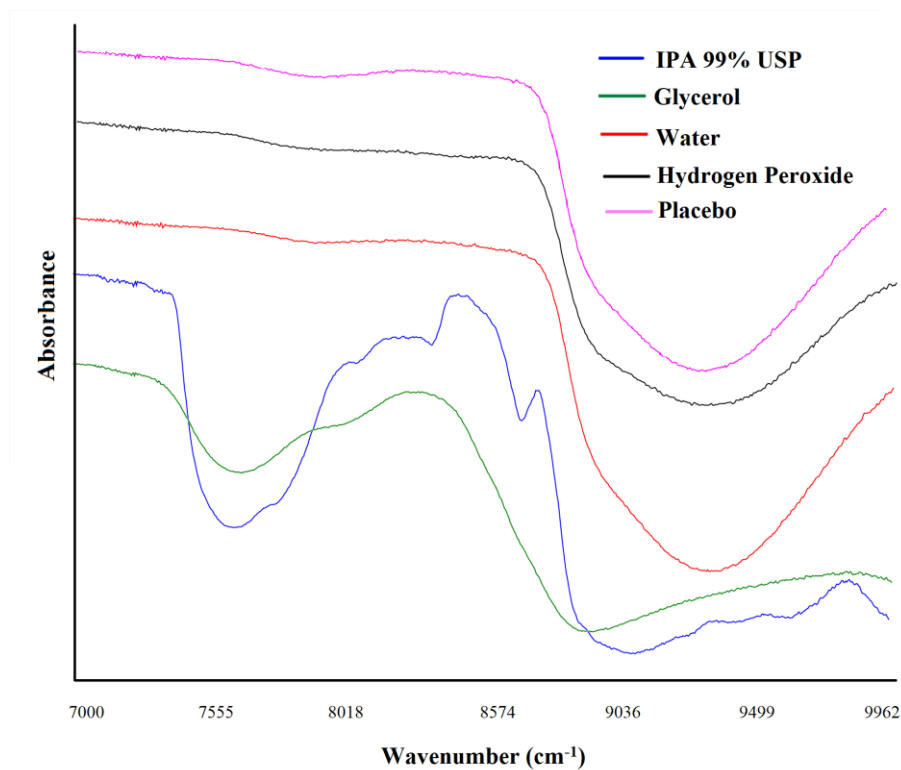
### **Statistical analysis**

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<sup>26, 27</sup>. The spectra of IPA displayed characteristic bands at 8176, 8415, 8716, 9846  $\text{cm}^{-1}$  and a trough at 7590  $\text{cm}^{-1}$ . On the other hand, liquid water showed absorption band at 9330  $\text{cm}^{-1}$ , and hydrogen peroxide exhibited absorption band at 9300  $\text{cm}^{-1}$  with broader trough. Glycerol peak was characterized by a shoulder at 8269  $\text{cm}^{-1}$  with troughs on both sides at 8874 and 7625  $\text{cm}^{-1}$ . 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.

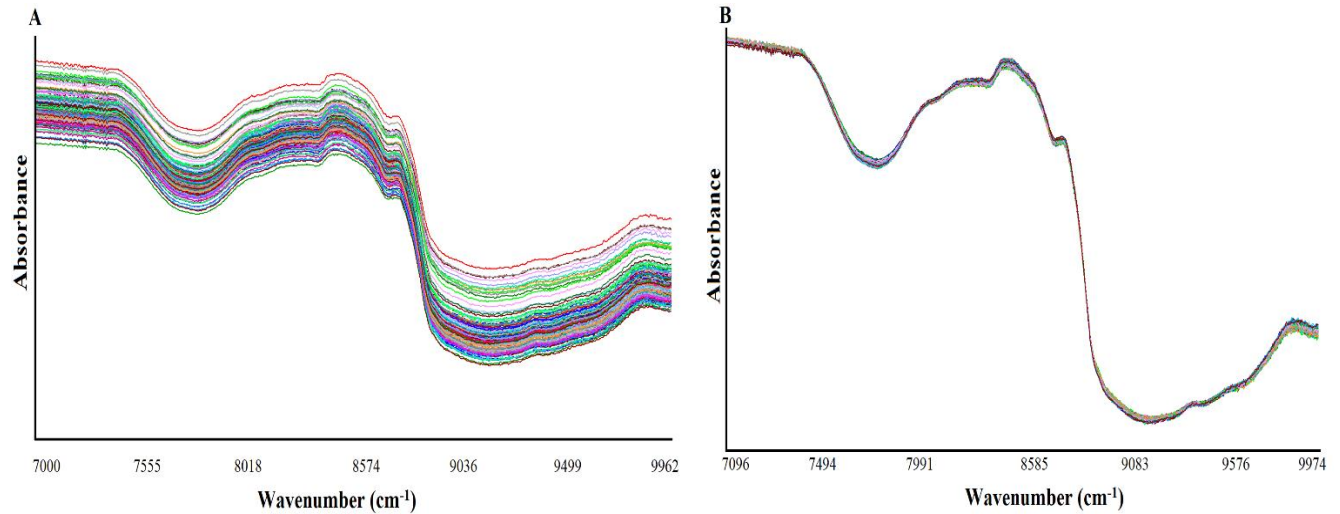


**Figure 1.** NIR spectra of IPA, water, glycerol, hydrogen peroxide and placebo sample

## Chemometric analysis

### Data processing

Truncated data of 7000-10000  $\text{cm}^{-1}$  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)<sup>23</sup>. 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<sup>19,20</sup>. 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**).

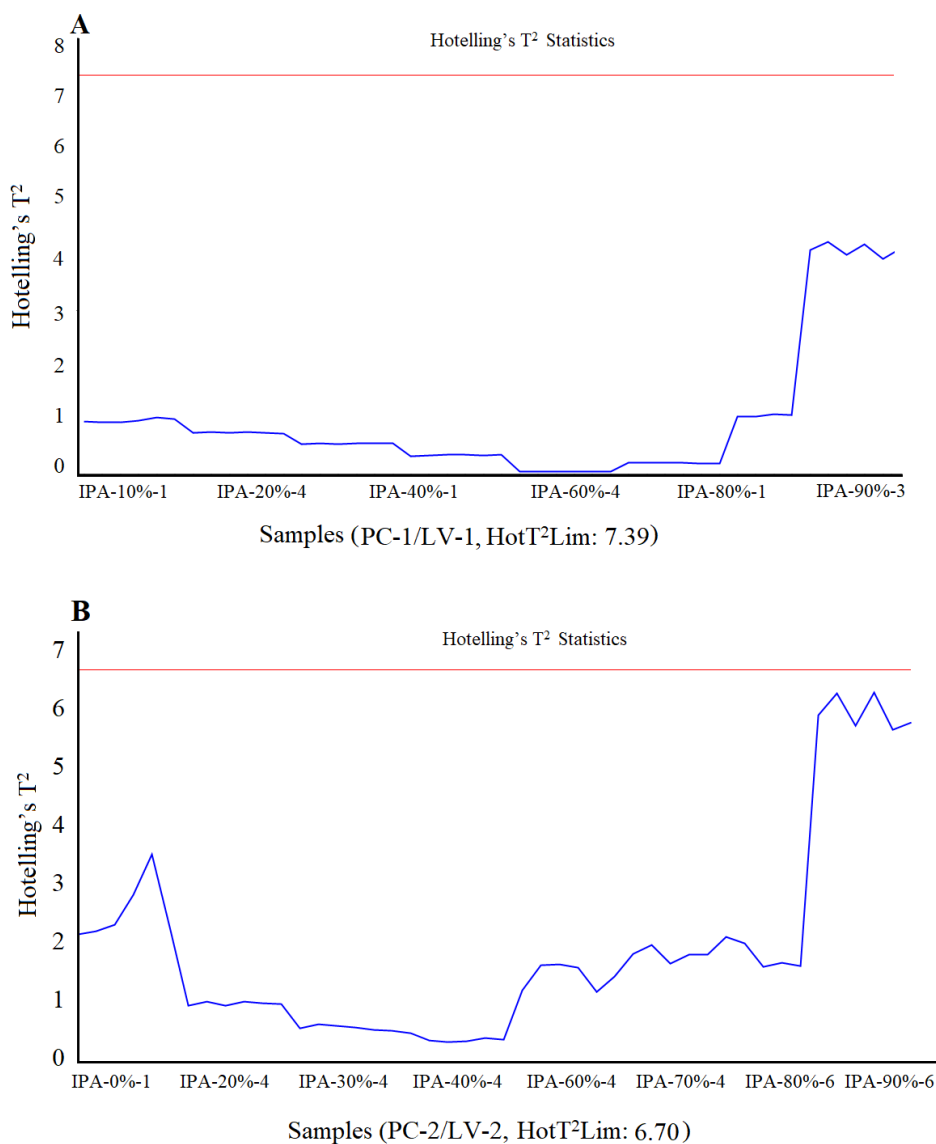


**Figure 2.** A) Truncated NIR, B) truncated NIR spectra pretreated with MSC

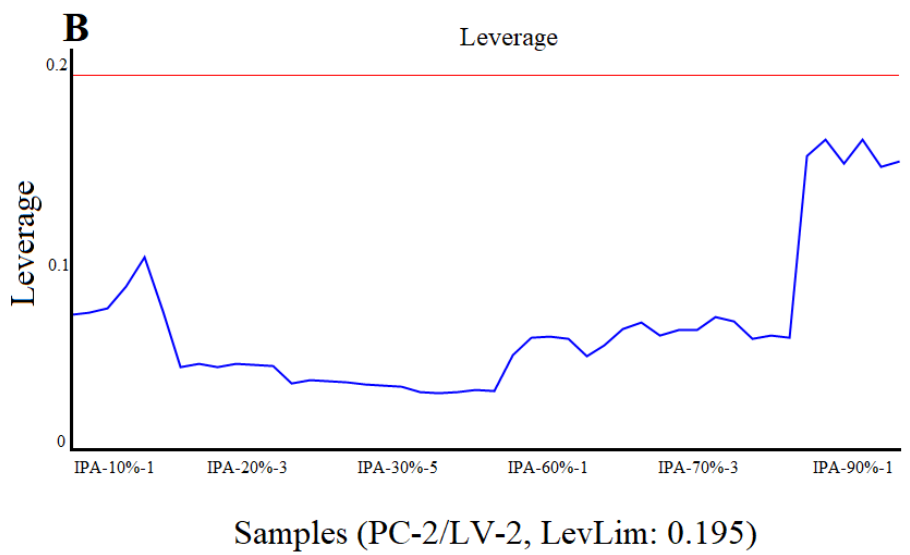
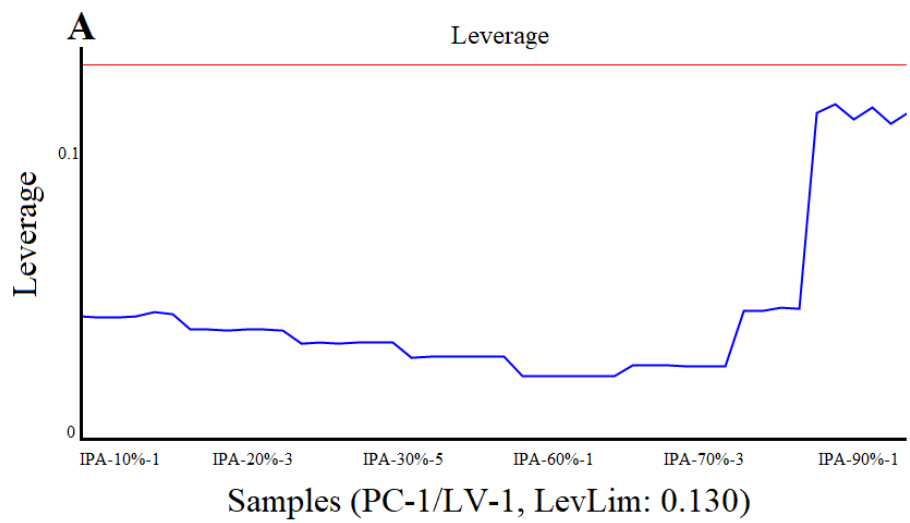
### Regression models

PCA and PLS are data dimensionality techniques. PLS combine features of PCA and multiple linear regressions<sup>28-30</sup>. 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<sup>31-33</sup>. Number of LVs used in this model were optimized based on statistical parameters determination coefficient ( $R^2_{CV}$ ) and root-mean-square error of cross validation (RMSECV)<sup>34, 35</sup>. 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 6.7 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). 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**). 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.

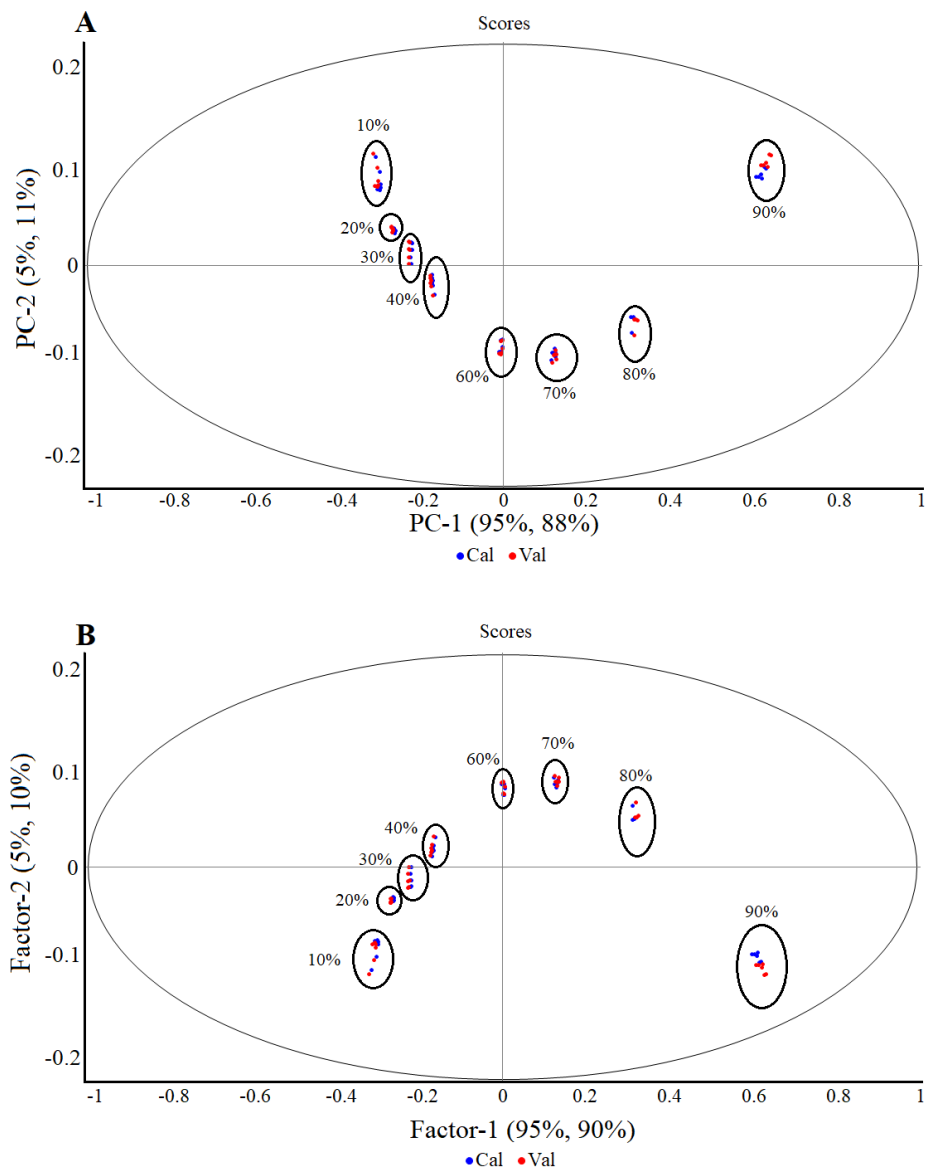


**Figure 3.** Hotelling  $T^2$  curves for A) PC-1/LV-1 and B) PC-2/LV-2.



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





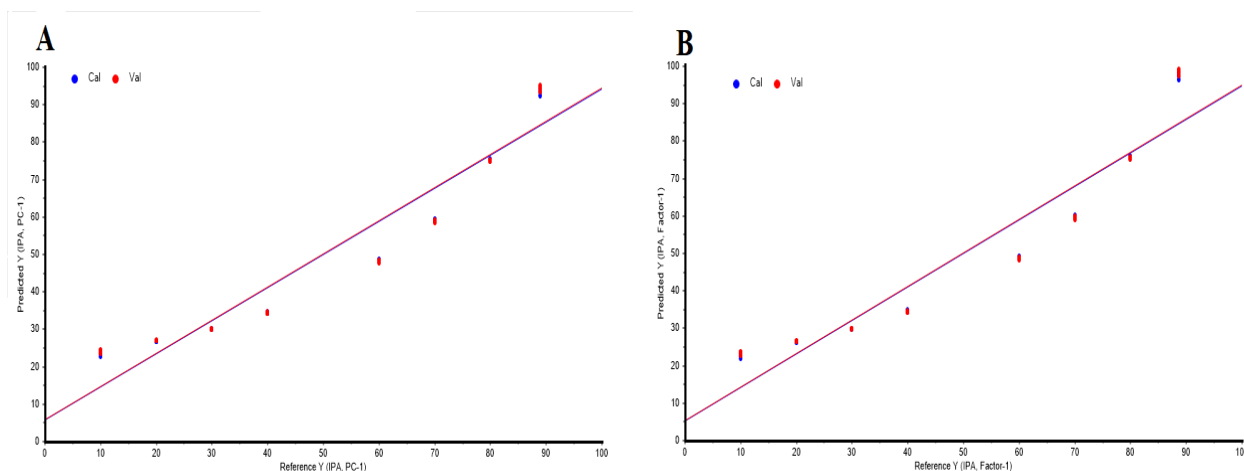
**Figure 5.** Score plots between A) PC-1 and PC-2 and B) LV-1 and LV-2

### Calibration and Internal validation

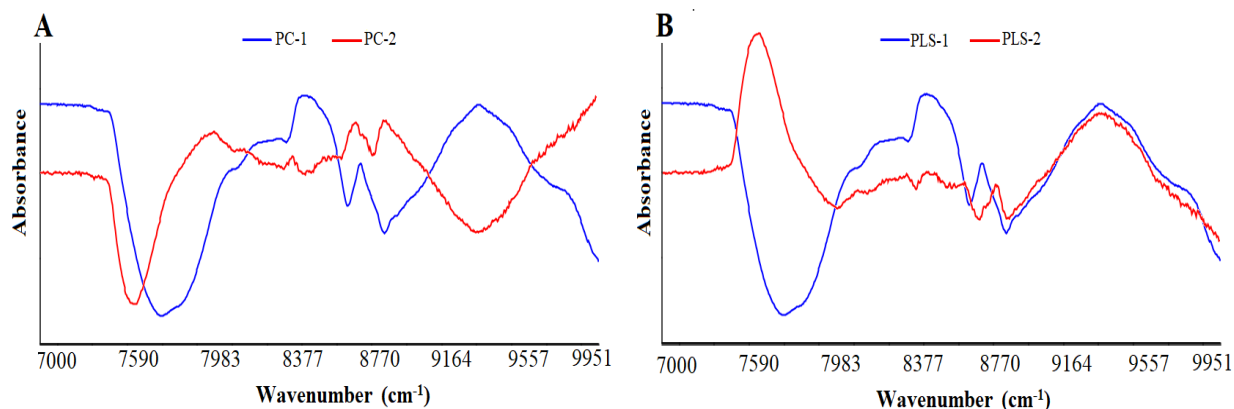
Initially, samples containing 0%-100% of IPA was used for model development with full cross validation wherein the same sample set of calibration models was used to validate the model. This is called internal validation. In this study, NIPALS algorithm was used for PLS and PCR regression models development<sup>36</sup>. 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<sup>19</sup> (**Table 1**). 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.

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<sup>-1</sup>. The PLS2 showed inverted peaks/valleys at 7536, 8369, 8709, 8805 and 9368 cm<sup>-1</sup>. 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 6.** Calibration and validation plots for A) PCR and B) PLSR



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

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

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

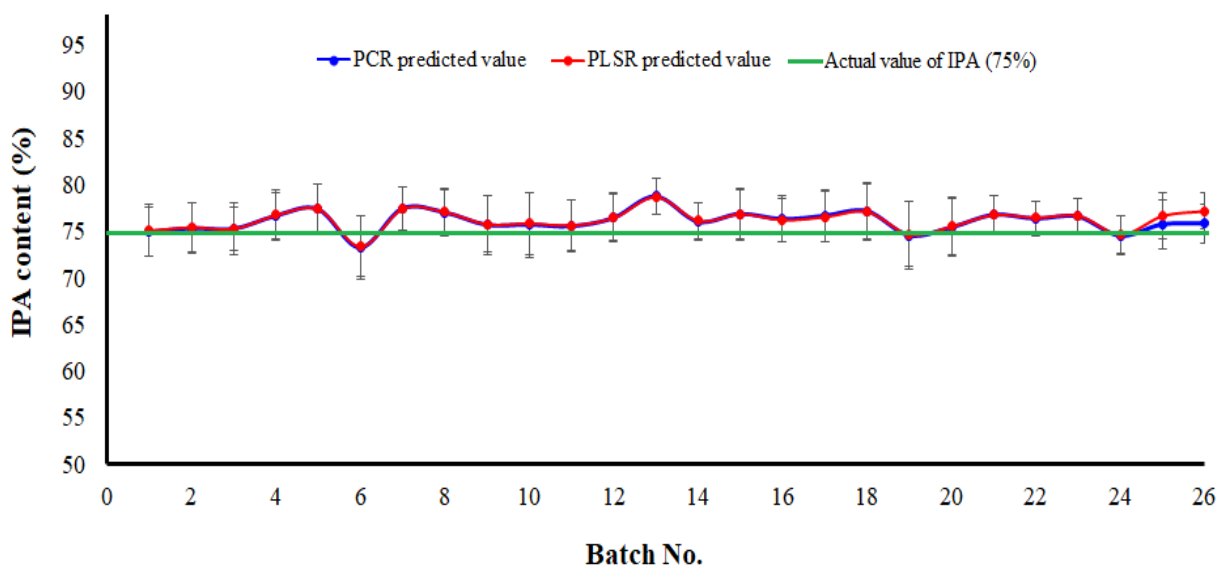
\*RMSEC (P) – Root mean square error of calibration or prediction;

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

### 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  $78.7 \pm 2.0\%$  with PCR and  $73.3 \pm 3.2\%$  to  $78.7 \pm 1.91\%$  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  $75.73 \pm 2.6$  and  $75.84 \pm 2.1\%$ , respectively. Similarly, the PLS predicted values for batch #25 and 26 were  $76.65 \pm 2.5$  and  $77.15 \pm 2$ , 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.

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**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 FB ALI: Analysis of the data

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