Rapid Discrimination of Halal and Non-halal Pharmaceutical Excipients by Fourier Transform Infrared Spectroscopy and Chemometrics

Nurul F. A. Razak¹, Roziah H. Abd Karim², Jamia A. Jamal³, Mazlina M. Said³

Introduction: The appendage of “halal” to a product is not just a guarantee that the product is permitted for Muslims, but it has also become favorable lifestyle choice globally. However, the expansion of halal pharmaceutical market was hindered by lack of global halal standards for pharmaceutical ingredients and product integrity analytical methodology. Objective: This work aimed to explore the possibility of using Fourier-transform infrared (FTIR) spectroscopy and chemometrics to develop multivariate models to authenticate the “halal-ity” of pharmaceutical excipients with controversial halal status (e.g., magnesium stearate).

Materials and Methods: The FTIR spectral fingerprints of the substance were used to build principal component analysis (PCA) models. The effects of different spectral pretreatment processes such as auto-scaling, baseline correction, standard normal variate (SNV), first, and second derivatives were evaluated. The optimization of the model performance was established to ensure the sensitivity, specificity, and accuracy of the predicted models. Results: Significant peaks corresponding to the properties of the compound were identified. For both bovine and plant-derived magnesium stearate, the peaks associated can be seen within the regions 2900 cm⁻¹ (C–H), 2800 cm⁻¹ (CH₃), 1700 cm⁻¹ (C=O), and 1000–1300 cm⁻¹ (C–O). There was not much difference observed in the FTIR raw spectra of the samples from both sources. The quality and accuracy of the classification models by PCA and soft independent modeling classification analogy (SIMCA) have shown to improve using spectra optimized by first derivative followed by SNV smoothing. Conclusion: This rapid and cost-effective technique has the potential to be expanded as an authentication strategy for halal pharmaceuticals.

KEYWORDS: Chemometrics, Fourier transform infrared spectroscopy, halal pharmaceuticals, magnesium stearate, principal component analysis, stearic acid

Original Article

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INTRODUCTION

Magnesium stearate consists of two equivalent fatty acids, namely stearic acid and palmitic acid, while magnesium acts as charge that will hold them together. This compound is available in the market with a few mixtures of crystalline forms such as anhydrate, monohydrate, dihydrate, and trihydrate.[1] Magnesium stearate is widely used in the production of dietary supplements, pharmaceutical tablets, capsules, and powders as well as many food products, including a variety of confectionery, spices, and baking.[2] It is also a well-known excipient in the pharmaceutical industry. The main function of magnesium stearate is

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as lubricant in solid dosage form drugs. A lubricant acts as an additive substance to reduce friction and to ensure that tablets are ejected properly from the press.[3]

Stearic acid and palmitic acid can be derived from animal or vegetable fats. The commercial sources of magnesium stearate are normally from animals, mostly porcine and bovine. Magnesium stearate is commonly categorized as haram or mushbooh in Islam because of the lack of guidelines available from the healthcare provider. Owing to the various sources of magnesium stearate, the authentication of this compound is crucial.[4] This becomes a major concern for consumers due to cultural and religious beliefs. For example, Muslims and Jew are forbidden to consume any products originated from lard and porcine, whereas no meat, eggs, fish, and mushroom for Hindus.[5]

In the meantime, having an efficient method to check the halal product integrity is crucial. Several methods such as chromatography-based methods, differential scanning calorimetry, electronic nose (EiNose) technology, DNA-based methods, and Fourier transform infrared (FTIR) spectroscopy showed potential in identifying halal and non-halal. Although most of these methods are too laborious, time-consuming, and expensive for routine analysis, FTIR can be a rapid and economical yet reliable method for this purpose. Attenuated total reflection-FTIR (ATR-FTIR) spectroscopy has been reported to be a reliable, accurate, and rapid technique that requires less than 2 min per sample analysis and minimum sample preparation.[6] This method is known to be nondestructive and able to provide information on the chemical and molecular properties of various substances.[7] FTIR spectroscopy can also be considered as “green analytical chemistry” as it reduces or eliminates the use of solvents and chemical reagents, which are hazardous to human health and environment.[8,9]

Although most of these studies are focused on the detection of lard or raw gelatin, or gelatin in processed foods, capsule, and dental materials, the possible use of this method in combination with chemometric application in halal pharmaceutical raw materials authentication is yet to be explored and established. Unlike food, the analysis of pharmaceutical products is rather more difficult due to the complex nature of the ingredients. As highlighted by the MS2424, the concept of halal in pharmaceuticals is focused on building “halal-ity” throughout the manufacturing process and beyond and not just depending on the end product testing. Therefore, there is a need for an efficient and effective method of identification and classification of halal and non-halal raw materials.

This study aimed to explore the possibility of developing this method and using it as a classification model to classify the halal and non-halal property of an important pharmaceutical excipient with highly controversial halal status—magnesium stearate. The fingerprint properties of the selected raw materials will be identified and compared, and this information will be used to build a classification model and database. This database will be a useful guidance for the industry to obtain the pharmaceutical ingredients of verified halal quality.

**Materials and Methods**

**Sample materials**

Magnesium stearate was obtained from different sources, which include Magnesium Stearate USP Reference Standard (Sigma–Aldrich, St. Louis, Missouri) labeled as (STD), Ligamed MF-2-K-Halal and Kosher grade (Peter Greven, Bad Münstereifel, Germany) labeled as (PG), MS-P/USP-Plant-derived (Sun Ace, Tanjong Penjuru, Singapore) labeled as (SA), and pharmaceutical grade (bovine-derived) obtained from local pharmaceutical manufacturers and online sellers labeled as (GB, LG, UPHA, PF). Each sample was divided into five sets, and each set was analyzed in triplicate. Samples were kept in air-tight containers and stored at room temperature.

**Sample preparation**

All samples were analyzed as it is, without sample preparation needed. The sample powder was placed centrally on the sample stage over the light beam in the ATR-FTIR instrument. For sample without valid identification certificate, the authenticity of these samples was verified using compendial methods.

**ATR-Fourier transform infrared spectroscopy analysis**

Spectroscopy analysis was conducted on PerkinElmer 100 spectrometer (PerkinElmer, Watham, MA, USA) equipped with an ATR accessory with ZnSe. ATR is a technique whereby the sample is placed in contact with the ATR element (ZnSe crystal, 45 ends). All spectra were recorded in the spectral range of 650–4000 cm⁻¹ with a resolution of 4 cm⁻¹ with 32 scans. The analyses were performed in triplicates, and the average spectra were used for multivariate analysis.

**Chemometric analysis**

Spectral preprocessing and chemometric analysis were conducted using the Unscrambler X software (Camo,
Oslo, Norway). Considering the lack of enough prior information concerning the measured spectra, different options were investigated to optimize data preprocessing. Principal component analysis (PCA) and soft independent modeling classification analogy (SIMCA) were used for multivariate analysis of the FTIR spectra.

Figure 1: Fourier transform infrared absorbance spectrum of magnesium stearate API of (A) kosher/halal standard, (B) bovine standard, and (C) vegetable standard in the mid-infrared region (4000–650 cm\(^{-1}\))
PCA models were initially constructed individually for each batch of the samples, followed by the formation of the PCA models for the whole spectral database. The outliers were determined using an automated approach and were removed before the analysis. PCA was conducted on all the data with leverage correction as

Figure 2: Spectral fingerprint differences between the (A) raw spectra, and post transformation with (B) baseline-correction, (C) smoothing by standard normal variate (SNV), and (D) first-order derivatives and SNV processing

Figure 3: Principal component analysis (PCA) score plot shows samples distribution and groupings based on PCA of spectra in the form of (A) raw spectra, (B) baseline-corrected, (C) smoothing by standard normal variate (SNV), (D) first-order derivatives and SNV processing. The red circle indicates the clustering of halal API on the positive side of the score plot
the validation method, and the scaling factor was set as 1. Leverage correction was used in this analysis as it is a simple method. Although this method is inferior to other validation approaches, it is a useful procedure for quick screening of unknown samples against diverse datasets. Classification analysis of validation samples was further conducted using SIMCA and the PCA models. A total of 32 samples from the calibration data were automatically selected for cross validation for each PCA and SIMCA models development.

RESULTS
The results of this study are present in Figure 1, Figure 2, Figure 3, and Figure 4.

DISCUSSION
ATR-Fourier transform infrared spectroscopy reflection
Figure 1 shows the FTIR spectra of magnesium stearate active pharmaceutical excipient (API) of kosher standard, vegetarian standard, and bovine standard with almost similar fingerprint profile. The spectra correlated with functional groups were observed on peak 2900 cm⁻¹ (C–H), 2800 cm⁻¹ (CH₃), 1700 cm⁻¹ (C=O) and 1000–1300 cm⁻¹ (C–O). There were almost no significant differences between the major peaks of the raw spectra that could be observed between the three different classes of samples. The raw spectra were subjected to optimization by selecting the appropriate preprocessing strategy to offer a reliable classification model. Figure 2 highlighted the presentation of the new fingerprints with differences within the same region for each class of samples after preprocessing. The algorithm of polynomial fitting was adopted to perform spectral smoothing to remove part of the random noise present in the signal and enhance the signal-to-noise ratio (SNR). Second derivatives were applied to enhance spectral differences and removed baseline and background. Because direct differencing tends to decrease the SNR by enhancing noise, the derivative spectra were also computed by polynomial fitting algorithms. Standard normal variate (SNV) was used to transform each measured spectrum into a signal with zero mean and unit variance. It was originally proposed to reduce scattering effects in the spectra but was also proved to be effective in correcting the interference caused by variations in pellet thickness or optical path.

Chemometric analysis
PCA was used to observe the classification of magnesium stearate between kosher, vegetarian, and bovine sources, as claimed by manufacturers. PCA is a chemometric application used to reduce the dimensionality of data to examine its underlying structure and the covariance/correlation structure of a set of variables. Although this method provides a simple means for identification of the principal components (PCs), solutions achieved may not possess certain desirable properties including robustness, smoothness, and sparsity. Reris and Brooks highlighted
the need of optimization strategy in providing an optimal output for PCA method. Spectral preprocessing played an important role to optimize separation between the samples in PCA. Figure 3 revealed the score plot of PCA using preprocessed spectral fingerprints of samples, defined by the first principle component (PC1) and the second principle component (PC2). Using first derivative spectra followed by SNV smoothing, preprocessing strategy had enabled samples from bovine (non-halal) and kosher/vegetarian (halal) to be clustered well in the score plot.

Validations samples from plant-derived magnesium stearate (SA and PG) were used to test the accuracy of the classification models. SIMCA analysis represented by the Cooman's plot in Figure 4 shows perfect classification for both sets of samples within accuracy level of 100%, meaning no samples were mistakenly classified into the wrong group. Misclassifications might take place due to close similarity of chemical composition, inappropriate selection of wave number region, and preprocessing method.

CONCLUSION

FTIR spectroscopy in combination with the chemometric application is a rapid and cost-effective technique that has the potential to be expanded as an authentication strategy for halal pharmaceuticals. The right preprocessing strategy will aid in the formation of a reliable classification model.

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Conflicts of interest

There are no conflicts of interest.

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