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Attenuated Total Reflection Fourier Transform Infrared Spectroscopy for Forensic Screening of Long-Term Alcohol Consumption from Human Nails

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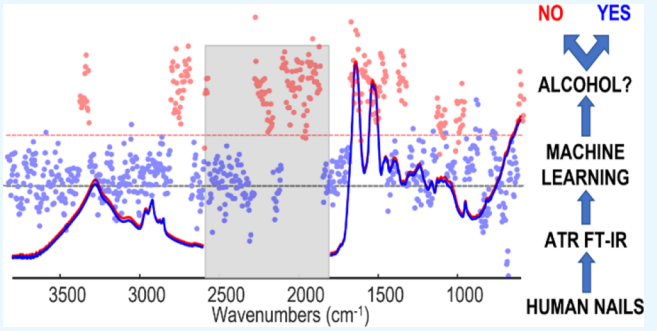
ABSTRACT: Fourier transform infrared (FT-IR) spectroscopy is used throughout forensic laboratories for many applications. FT-IR spectroscopy can be useful with ATR accessories in forensic analysis for several reasons. It provides excellent data quality combined with high reproducibility, with minimal user-induced variations and no sample preparation. Spectra from heterogeneous biological systems, including the integumentary system, can be associated with hundreds or thousands of biomolecules. The nail matrix of keratin possesses a complicated structure with captured circulating metabolites whose presence may vary in space and time depending on context and history. We developed a new approach by using machine-learning (ML) tools to leverage the potential and enhance the selectivity of the instrument, create classification models, and provide invaluable information saved in human nails with statistical confidence. Here, we report chemometric analysis of ATR FT-IR spectra for the classification and prediction of long-term alcohol consumption from nail clippings in 63 donors. A partial least squares discriminant analysis (PLS-DA) was used to create a classification model that was validated against an independent data set which resulted in 91% correctly classified spectra. However, when considering the prediction results at the donor level, 100% accuracy was achieved, and all donors were correctly classified. To the best of our knowledge, this proof-of-concept study demonstrates for the first time the ability of ATR FT-IR spectroscopy to discriminate donors who do not drink alcohol from those who drink alcohol on a regular basis.



Fingernail and toenail clippings are a capable and alternative matrix in providing drug monitoring research. The current literature has established the presence of drug biomarkers because of the analysis of the chemical composition of fingernails and toenails. The keratin structure of fingernails and toenails allows for the long-term presence and incorporation of drugs in nails.[1] As the fingernail continues to grow, the germinal matrix is consistently receiving and incorporating any biomarkers to the overall chemical composition of the fingernail.[1] Thus, allowing for the detection and identification of exposure. The accumulation of biomarkers within the nail allows for the determination of long-term consumption or exposure.

Current research has found long-term alcohol biomarkers that result from the drug distribution over the entire fingernail.[2] In criminal justice and from a civilian perspective, the fingernail analysis of drug or alcohol monitoring would play an important role in probation, critical jobs, and at the minimum to know the habits of an unknown offender.

The anatomy and physiology of fingernails make them an excellent matrix for the retention of alcohol biomarkers specifically. The fingernail's keratin composition allows for a variably strong yet porous surface that allows for the incorporation of drugs or chemicals.[3] Fingernails and toenails' chemical composition remain unaffected when drugs or chemicals are introduced into the nail as it continues to grow.[4,5] Thus, as alcohol consumption continues, the alcohol biomarkers will accumulate in the keratin fibers for months showing history of use.[6]

Research involving fingernail and toenail chemical analysis includes disciplines from dermatology to criminal justice. The microscopic physical striations of fingernails have also been explored and compared as ridge patterns.[7] A fingernail's appearance and physical composition may provide insight into nail diseases, mineral or vitamin deficiencies, poor nutrition, and recent trauma.[3] Exposure and contaminants such as poison, chlorine, and explosives have also been mentioned within the literature.[3,4,7−10] Particularly, alcohol biomarkers found in the body after the consumption of alcohol may be

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present as aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), or carbohydrate deficient transferrin (CDT) biomarkers, to name a few. However, the particular alcohol biomarker of interest in monitoring alcohol abuse is ethyl glucuronide (EtG), considering it is a resulting product only when ethanol is being metabolized in the liver.[1,11]

Previous studies found that fingernail analysis was able to identify acute and chronic drinking behavior.[1,12] EtG concentrations found in fingernails showed a correlation with self-reported alcohol consumption.[12] Especially in comparison to alternate hair samples, fingernail EtG concentrations were higher, concluding that fingernails provide a better alcohol biomarker.[12]

Thus, finger- and toenail analysis is a great specimen in analyzing alcohol markers especially for identifying long-term alcohol abuse, providing 3 - 6 months of alcohol use history. Using these specimens would benefit the monitoring of alcohol consumption of drug users/dealers, postmortem toxicology, drug-related crimes, and other forensic-related compliances, testing, and detection.[6]

Alcohol biomarkers are one of many drug markers which have been found in fingernail clippings, including parent drug and or metabolites of tobacco, marijuana, THC, opiates and opioids, nicotine, PCP, Zolpidem, Bromazepam, Benzodiazepines, Amphetamine and Methamphetamine, Cocaine, and Benzoylecgonine.[1,3,6,13−19] Research pertaining to understanding the incorporation of drugs or chemicals during nail formation have explored various methods of ingestion, including in utero drug exposure by analyzing infants' fingernails.[13,16,19]

Thus, nail analysis may be performed for alcohol and drug treatment to forensic toxicological analysis in drug-facilitated sexual assaults and postmortem cases, where there may have been a single distant exposure or unspecified drug use history that may provide information on the cause or manner of death.[1]

Other testing specimens are at the forefront of current forensic toxicology methods regarding alcohol biomarkers, including hair and bodily fluids such as urine.[1] While these testing specimens have become common practice, there are limitations and biases that have pushed for an alternative specimen.[1,11] Limitations regarding detection of alcohol biomarkers in other specimens are addressed with fingernail testing.[1] Studies comparing EtG concentrations of both fingernails and hair have concluded that concentrations are higher in nails.[1,12] Research has attributed these results to the fact that fingernails lack pigment, so concentrations are not completely detected and or are degraded by cosmetic treatments like bleaching and dyeing.[1,6] In comparison to other specimens like blood, urine, and saliva, fingernails exhibit a longer window of detection, noninvasive sample collection, lack of drug degradation, and require a smaller sample for analysis.[14,20]

Techniques utilized for detection of substances in hair and nails include gas chromatography (GC), mass spectrometry (MS), nuclear activation, X-ray fluorescence and emission, and atomic absorption and emission.[4] Additionally, techniques used to examine nail are as follows: laser-induced breakdown spectroscopy; high-performance liquid chromatography (LC); ultraperformance liquid chromatography−tandem mass spectrometry (LC−MS); micro-PIXR and micro-RBS; hard X-ray micro-analysis; and synchrotron-based XFR.[7,14] Following the validation of a liquid chromatography tandem mass spectrometry (LC−MS/MS) technique, studies on fingernail EtG concentration testing have continued with this method of detection.[6] The evaluation of EtG detection regarding sensitivity and specificity with the LC−MS/MS method is yet to be assessed. However, current studies with an inadequate number of samples have shown a correlation between the detection of alcohol intake and EtG values.[6]

Drugs of abuse and pharmaceuticals in nails are present only in low concentrations. Because of the complexity of the keratinized matrix, analytical methods need to be more sensitive, and sample preparation is essential.[14] Fourier transform infrared (FT-IR) spectroscopy has been utilized as an analytical technique for numerous applications in the forensic field. FT-IR spectroscopy has been applied to a wide range of types of evidence, and it performed well in the examination of questioned documents found at a crime scene,[21] paints,[22] banknotes,[23] fibers,[24] hair,[25,26] gunshot residue analysis,[27] and body fluid traces.[28−31] The nondestructive nature of this method enables us to utilize the same portion of the sample for further examination. This method is rapid, nondestructive, and quantitative, which are ideal properties for forensic analysis. Moreover, this method can be used in the field with a portable instrument.[32] IR spectroscopy belongs to analytical methods that are capable of molecular confirmation based on Standard Practice for Identification of Seized Drugs that guides forensic laboratories in the identification of an unknown seized substance.[33] FT-IR spectroscopy was widely studied and has already been utilized in forensic casework. Abusing ethanol can often lead to negative consequences such as criminal prosecution or civil lawsuits.[34] Because this methodology has also proven itself in the seized drug analysis for obtaining information on the chemical composition of the sample,[35−39] it would be highly favorable to apply this technique in other fields of forensic investigation, such as alcohol and its metabolite detection.

FT-IR was successfully used for human fingernail analysis by Coopman et al. to study the concentration of glycated nail proteins[40] and whether the glycation reflects the average glycemia over the last months. To the best of our knowledge, the only study using FT-IR to study human nails for forensic purposes is a study of Sharma et al. that used partial least square discriminant analysis (PLS-DA) to classify sex by chemometric analysis applied to the spectral data of ATR-FT-IR spectroscopy of human fingernail clippings.[5]

In this study, we describe the development of a novel method of ATR FT-IR spectroscopy coupled with PLS-DA. PLS is a well-known dimension-reduction algorithm that is a simple and efficient method because the information about a class assignment is directly involved in the extraction of latent variables (LVs). The PLS algorithm is a linear regression method that can be used for three different scenarios: data exploration, calibration, and classification. When the dummy response array for categorical outcomes is used instead of the usual continuous vector, the PLS model is called partial least squares discriminant analysis (PLS-DA).[41] The goal in our research was to evaluate the applicability of human nail samples for ATR FT-IR as an alternative to other techniques for the monitoring of long-term alcohol consumption. Specifically, we were interested in whether the method has the potential to differentiate people who are drinking ("Alcohol drinkers") or not drinking alcohol ("Non-drinkers") based on their nail clippings.

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MATERIALS AND METHODS

Sample Generation and Preparation. Nail clippings were collected from 63 volunteers (44 alcohol and 19 nonalcohol donors) of diverse races and ages. The eligibility criteria for volunteers were healthy nails with no distinct symptoms of skin or nail disease. Regarding the alcohol drinking status, if they consume alcohol, they were required to drink within the past six months before nail clipping collections and at least 2 standard drink equivalents per week. This ensured that alcohol consumption can be correctly detected in nail clippings regardless of whether the specimens come from fingernails or toenails. Samples consist of 1−10 donated nail clippings from each volunteer, with the length at least 1 mm. Each sample was cleaned with 70% isopropyl alcohol and stored in zip-lock bags and coded. The study was approved by the Institutional Review Board of Texas Tech University (No. IRB2022-211).

FT-IR Spectroscopic Analysis. The spectra were recorded on a Thermo Fisher Nicolet iS10 equipped with a diamond ATR accessory. The acquisition of all spectra was carried out by 32 scans per spectrum and in the range 4000−600 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were collected from different spots of the sample to count for heterogeneity of the nail matrix. A background spectrum was recorded before collection of the spectrum of each new sample, and it is subtracted from all subsequent spectra. Measurements were controlled by OMNIC software (Thermo Nicolet Corporation, Waltham, MA, USA). Between each sample analysis, the crystal was cleaned using isopropanol and allowed to dry before starting measurements. To ensure reproducible contact between the sample and the crystal, a force is applied by pressure gauge on the nail sample to provide good optical contact with the crystal. The spectral ranges of 600−1800 and 2600−3800 cm^{-1} showed contribution from biochemical composition of the samples and were used for further analysis.

Statistical Analysis. The OMNIC spectral (.SPC) files were imported into other software for further processing and statistical analysis. The PLS-DA modeling was performed using the software MathWorks MATLAB R2020b version 9.9.0.1570001 (Natick, MA, USA) supported by the Eigenvectors Research Inc. PLS Toolbox 9.0 (Manson, WA, USA). The sparse PLS and ROC analysis was performed using R software, the R package "mixOmics",[42] and package "pROC".[43] The following pre-processing steps were applied: transformation of the transmission to absorbance ($\log(1/T)$), second-order derivative with the second polynomial, and normalization by the total area and mean centering. The preprocessing steps were selected on a training data set. Spectra in the test data set were preprocessed right before their predictions.

PLS algorithm is responsible for reducing the dimension of data (i.e., FT-IR spectrum) into a few LVs in a supervised manner, and then, a threshold is applied to assign class labels to a spectrum (Alcohol drinkers vs Non-drinkers). PLS components are selected to maximize covariance between the responses (class assignment) and a new linear combination of the original predictors. Then, the final dimensionality is determined by deciding how many of these new predictors to include in a model.

The first step for PLS-DA multivariate analysis was the selection of donors whose spectra will constitute the calibration and test data sets. For comparison purposes, several PLS-DA models were built with increasing numbers of spectra in training and validation data. During each split, we always kept the spectra from one donor either in the training or test data set. Each model was built with the optimal number of LVs. When the trained model showed lower variance during the training process, that data split was selected. The samples were split into the calibration data set (training) and the test data set (external validation) corresponding approximately with 80:20 split. The donors were assigned to the calibration and test sets randomly; their final distribution was examined in the LV projection hyperspace to ensure that datasets are representative samples (e.g., random sample) of the original dataset (Figure 1). The training data set consists of 669 spectra from 51 donors and validation data set consists of 162 spectra from 12 donors.

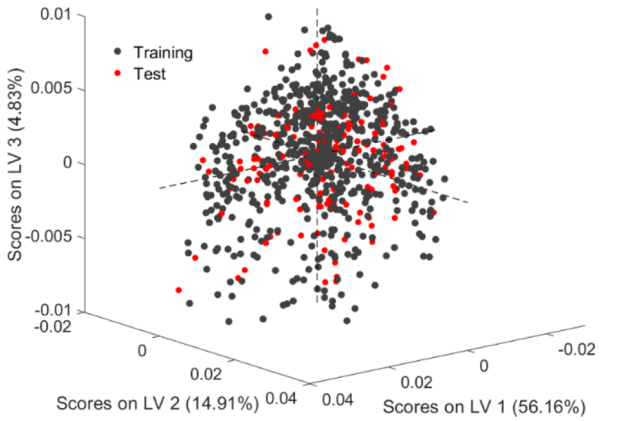

Figure 1. Calibration set and test set are both random samples of donors to prevent any biases in the dataset retained in the samples.

Subsequently, cross-validation was applied to the selected training data set for tuning PLS-DA parameters; thus, there was no standalone validation set. The only parameter to be optimized is the number of LVs that should be included in the model. The selection of the final model complexity e.g., number of LVs as well as an estimate of the classification error rate of the PLS-DA model were obtained using a single 10-fold venetian blind cross-validation on the training data set.[44] Ten-fold CV is a good compromise in terms of the bias-variance trade-off.[45] In the PLS-DA context, in each step, 1/10th of the training data set was left out and the remaining spectra were used to train a PLS-DA model. This model was used to predict the class label, e.g., Alcohol drinker or Non-drinker on the left-out spectra. This process was repeated with the next 1/10th till all spectra were predicted. All spectra were left out for prediction only once. The number of LVs that yielded the lowest classification error rate during the CV was selected to be used for the final PLS-DA model.[46]

In the next step, prediction was performed on the samples of the test data set to externally validate our model. The evaluation of the prediction ability of the models was assessed while using two evaluation parameters: the classification accuracy, with its corresponding sensitivity and specificity and the Area Under the curve of the Receiver Operating Characteristic (AUROC). Accuracy and AUROC are suitable parameters to assess the quality of PLS-DA models that discriminate between two classes.[47]

Sparse PLS (sPLS) variable selection was used to select relevant spectral regions for differentiation of the two classes.

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For the case of multiple highly correlated variables in the data set, such as FT-IR spectra, extension of a sparse PLS exploratory approach was proposed and used here to perform variable selection in a classification framework since this method proved itself that it is competitive to other methods in terms of interpretability and computational time.[48] sPLS-DA performs variable selection and classification in a one-step procedure; specifically, sPLS forces sparsity of the loading vectors by imposing L1 penalty with a tunable sparsity parameter, thereby the subsequent LVs depend only on a subgroup of the original group of predictors.[48] This step was performed primarily to understand what FT-IR bands contribute the most to the final model.

RESULTS AND DISCUSSION

The purpose of this study was to apply ATR FT-IR spectroscopy as a nondestructive method for differentiating Alcohol drinkers and Non-drinkers based on human nail clippings. The experimental design was chosen based on the literature and preliminary results. The nail clippings make an excellent specimen. They make a reservoir matrix made up of a tight connection of keratin fibers: nails are also porous and thus are an excellent matrix for trapping and catching alcohol biomarkers. To collect nail specimens is a simple and noninvasive process, and they are easy to store at room temperature.

The average ATR FT-IR spectra of human nails obtained from two different groups of volunteers are shown in Figure 2.

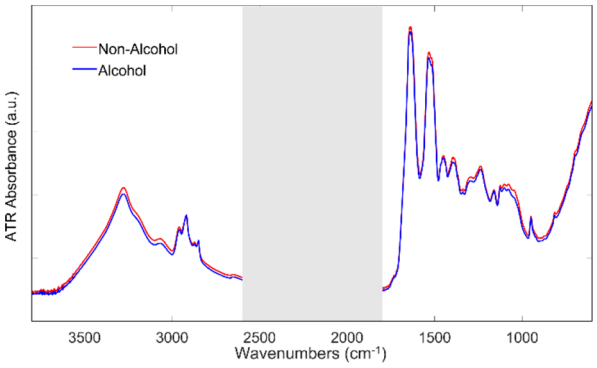

Figure 2. Averaged raw spectra of nail clipping for the group of Alcohol drinkers (blue line) and Non-drinkers (red line). Spectral ranges 3800−2600 and 1800−600 cm^{-1}, depicted in figure, were used for further statistical analysis.

The ATR FT-IR spectra of the two groups are very similar, showing the same bands of similar intensities. A differentiation based on visual inspection would be impossible; hence, the advanced multivariate statistical analysis was required for our goal. The spectral signature of human nails shows vibrational frequencies of different biomolecules and provides information about these key components present in human nails. Nails consist mostly of fibrous proteins generally called keratin. Keratins are long chains of amino acids linked together with amide bonds.[52] In the nail IR spectra, major regions can be identified; in addition to proteins, nail spectra show contribution from nucleic acids (1000−1250 cm^{-1}), lipids (2800−3000 cm^{-1}), and carbohydrates (1000−800 cm^{-1}).[49,53] Table 1 shows the peak assignment of molecular vibrations that were available from the literature.

Table 1. IR Spectral Peaks in Human Nails and Their Assignments

wavenumber (cm^{-1})	component identification	band assignment	ref.
3278	amide A	N−H stretching	49
3067	amide A	N−H stretching	5
2961	lipids and proteins	C−H asymmetric stretching of CH_3	50
2920	lipids and proteins	C−H symmetric stretching of CH_3	50
2850	lipids and proteins	C−H symmetric stretching in CH_2	50
1637	amide I	C═O stretching	50
1533	amide II	C═O stretching coupled with C−N stretching and bending deformation of N−H	50
1452	lipids and proteins	CH_2, CH_3 asymmetric bending modes	5,50
1396	amino acids	symmetric mode of CH_3	51
1296	amide III	N−H bending	5
1239	amide III, nucleic Acid	C−N stretching, asymmetric stretching mode of PO_2^-	5,50
1080	nucleic Acid	symmetric stretching of PO_2^-	50

The PLS-DA method was applied to develop a robust model for the identification of Alcohol drinkers with respect to the spectral signature approach. That means, rather than looking for the differences in the intensities of individual peaks, we seek for regularities in spectral signatures of known labeled examples, train a system to identify learned features and patterns, and make subsequent predictions on unknown data. The donors forming the calibration and the test data set matrixes were categorized as "Non-alcohol" (19 donors) and "Alcohol" drinkers (44 donors), as explained in the definition provided above. The dataset of 669 "labeled" spectra was used to develop a PLS-DA model, which was cross-validated using 10-fold Venetian blind to determine the optimal number of LVs. Twelve LVs were determined during CV, when subsets of spectra were set aside and predicted, and subsequently, all predictions were averaged. We achieved the sensitivity of 97% and a specificity of 98% to differentiate Alcohol drinkers from Non-drinkers. After we established a PLS-DA model with twelve LVs for differentiation of the two groups of donors of nail specimens, we predicted on the test set, which we did not use for training the model. This allowed us to determine the out-of-sample error for the model. Figure 3 shows the class prediction for each spectrum together with the classification threshold (the red dashed line). The results from the training process are shown on the left (spectrum 1−669) and results from the external validation are shown on the right of the figure (spectrum 670−831). Any spectrum located above the threshold is predicted as "Non-alcohol", and any spectrum below the classification threshold is assigned as the "Alcohol" spectrum.

The external validation on the test data set resulted in sensitivity of 90% and a specificity of 92% to detect long-term alcohol consumption prior to nail collection. The PLS-DA model showed an accuracy of 100% for differentiating the two groups based on the donor level when standard 50% threshold is used, which means that no donor was misclassified. The classification model performance was also visualized by the plot of ROC curve that shows how the sensitivity changes with specificity while the threshold is varied. The obtained AUROC value is 0.97 (95% confidence interval [CI], 0.95−0.99),

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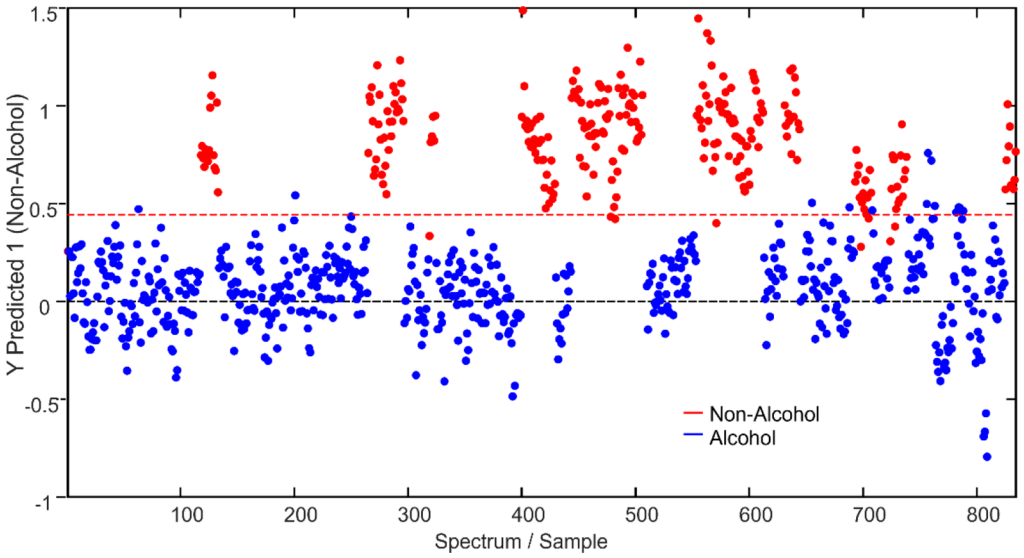

Figure 3. Calibration and external validation prediction results for the Alcohol drinkers and Non-drinkers based on human nail samples. Each point represents individual ATR FT-IR spectrum. The red dots denote the non-alcohol group, the blue dots represent the alcohol group, and the red dotted line denotes the classification threshold.

indicating that Alcohol drinkers and Non-drinkers groups are well separated with our PLS-DA classification model (Figure S-1).

Supervised PLS algorithm usually performs well in terms of classification test errors than competing methods; however, they do not always produce a sparse model involving a small number of features that perform better, and additionally, the highly correlated features may be a great deal of redundancy in the selected features.[45] Regions selected by sPLS correspond with wavenumbers with high contribution for the PLS model. The sPLS was able to reduce the number of variables, but still more than 1500 variables were left in the model, specifically 1562 variables. Such results suggest a complex relationship among nail-metabolic fingerprinting and environmental/behavioral factors as targeted in this project. Figure 4 shows

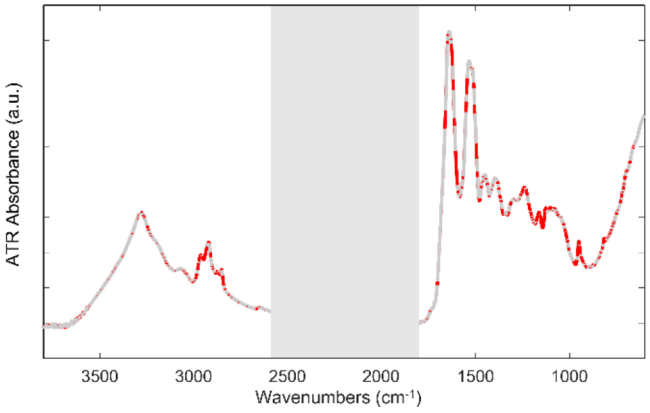

Figure 4. Average raw ATR FT-IR spectrum of human nail clipping with highlighted spectral regions selected by the sPLS method.

the most informative spectral regions for our goal and within the most frequently selected regions, the CH_2 and CH_3 groups of lipids and protein, Amide I, Amide II, Amide III bands are contributing the most to the model. Additionally, to these bands, the most wavenumbers of the spectral range between 950 and 1180 cm^{-1} were selected, and it should be mentioned that these parts of the IR spectrum of nails have been studied in connection with diabetes.[40,54]

The sPLS-DA model using only variables selected by the feature selection was cross-validated, and seven LVs were determined during CV. Both sensitivity and specificity achieved 90% determined to differentiate Alcohol drinkers from Non-drinkers. The external validation on the test data set resulted in a sensitivity of 83% and a specificity of 90% to detect long-term alcohol consumption prior to nail collection based on the sPLS-DA model (Figure S-2). The AUROC analysis showed a value of 0.96 (95% confidence interval [CI], 0.95−0.97) during CV and 0.93 (95% confidence interval [CI], 0.90−0.97) for external validation on the test data set (Figure S-1). Thus, when using the reduced subset of features for classification, the results from external validation decreased only slightly; however, a much more significant deterioration occurred during CV after the sparsity had been applied to the model. The relevance of features does not always imply optimality in the sense of the maximal accuracy produced by the classifier.[55]

The testing data set was used to assess the performance of the sPLS-DA model and also ensure that it can generalize well to new, unseen spectra. At this time of the process, we can compare the testing accuracy against the validation, or better against the training accuracy to ensure that the model was not overfitted.[56] The statistics for the training process showed the same sensitivity and specificity of 90% as during the CV. Both, training and CV accuracy do not significantly outperform testing accuracy, which means that the corresponding predictions from training, cross-validation and external validation set predictions are comparable, indicating that the model has not been overfitted.

To the best of our knowledge, this proof-of-concept study demonstrates for the first time the ability of ATR FT-IR spectroscopy to discriminate donors who do not drink alcohol from those who drink alcohol on a regular basis, and that is already at the level of drinking of two standard drinks a week. Nails grow at a slower rate compared to the similar matrix, hair (3 mm/month for fingernails and 1 mm/month for toenails); as a result, different alcohol markers, such as EtG, can better

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accumulate in the nail matrix and can enable taking advantage of the long diagnostic time window.[14]

Our proposed methodology can have implications for legal or clinical decision-making. There are several markers that can be used for screening of alcohol abuse in the long term. Each has some specific characteristics, making them suitable for different situations and methodology. Unfortunately, in general, alcohol markers in human nails are not well studied. EtG, mentioned above, is a direct marker of prolonged alcohol consumption in nails and also of overall drinking amounts. Until now, only two markers were established into practice for alcohol abuse detection in hair (an alternative matrix to the nail sample) and in the nail. In addition to the aforementioned EtG, another marker that had been studied in hair samples are fatty acid ethyl esters (FAEE). Both FAEE and EtG are direct alcohol markers because they still contain the ethyl group of consumed ethanol.[57] However, one of the significant advantages of this methodology, i.e., the ATR FT-IR spectrosopy combined with the ML approach as a diagnostic tool is that the spectra generated from human nails or any other biological matrix encompass not only the known and described markers. ML introduces here an automated spectroscopic data analysis of the entire vibrational fingerprints that are inherently complex and heterogeneous, but ML is capable to learn the pattern that is different between people who drink alcohol from those who do not drink alcohol. The patterns include also changes in spectral bands, which are not yet explained and well-studied in terms of biochemical components. However, our methodology makes it possible to measure all simultaneous changes in spectral profiles and thus provide high fidelity redundant multiple biomarkers of different specificity recognition at once.

CONCLUSIONS

There is a continuing need to explore and develop new technologies and ensure that the best and most accurate outcomes in both medical diagnostics and forensic science can be achieved. Human nail clippings are among the most practical specimens, because of their stability and rapid sampling. Additionally, the nail clippings do not suffer surface interference as do other trace evidence such as body fluids and latent fingerprints.

The major advantage of ATR FT-IR is a specific spectral signature for different sample groups based on their biochemical composition. The infrared spectrum displays unique vibrational characteristics of a sample based on the different absorption frequencies of the individual functional groups.[26] MLs are capable of elucidating multiplex spectral information, including spectral composition and the small differences caused by donor alcohol consumption status. The PLS-DA models could clearly differentiate the Alcohol drinkers' and Non-drinkers' nail clipping samples. Both PLS-DA and sPLS-DA models have shown excellent AUROC of 0.97 and 0.93, respectively, to discriminate between the two groups. Thus, our proposed method represents the development of a rapid analysis of nail specimens for forensic or medical purposes based on an ML approach applied on ATR FT-IR spectral data. Further analytical applications for other personal characteristics are feasible and, together with interferences of cosmetic treatments, are under investigation in our laboratory.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02579.

Trade-off between specificity and sensitivity from the PLS-DA model and the sPLS-DA model presented as an ROC curve and corresponding AUROC; calibration and external validation prediction results of the sPLS-DA model for the Alcohol drinkers and Non-drinkers based on human nail samples (PDF)

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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