K74 Developing a Raman Microspectrophotometric Method to Quantitate Carboxyhemoglobin (COHb) in CO-Exposed Blood Samples

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Learning Overview: After attending this presentation, attendees will understand how Raman microspectrophotometry may be used to quantitate the COHb concentration of blood samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a novel method for COHb quantitation using Raman microspectrophotometry that is comparable with established spectrophotometric methods while eliminating the need for time-consuming sample preparation and limiting the use of consumables and the generation of waste.

COHb quantitation is a routine toxicological analysis performed in cases of acute carbon monoxide poisoning. Quick and easy-to-use methods of COHb quantitation include spectrophotometry and CO-oximetry, although these methods can be inaccurate and imprecise both at low concentrations and in putrefied postmortem specimens. More reliable gas chromatographic quantitation methods are available as well, but these require extensive sample preparation and, therefore, require elevated use of consumables and increased analysis time. The goal of this study was to develop a Raman microspectrophotometric COHb quantitation method and assess its precision and accuracy in estimating the COHb concentration of ten experimental unknown samples that were subsequently analyzed using an Ultraviolet (UV) -visible spectrophotometric method.

A Thermo Scientific™ DXR2 Raman microscope equipped with a 785nm laser was used to determine the COHb concentration of the samples analyzed for this study. Stock solutions of 0% and 100% COHb were prepared by bubbling ultra-zero air and carbon monoxide, respectively, through whole human blood (healthy living donor, gender not specified) containing K_2EDTA anticoagulant for 30 minutes. A total of eight calibrator sets ranging from 0%–100% COHb at 10% increments were prepared via appropriate dilution of the stock solutions. These calibrators were deposited in 20-μL aliquots onto aluminum foil-covered microscope slides, which were then subsequently dried in a fume hood for at least one hour prior to analysis. Spectra were acquired at ten separate locations on each sample, and these spectra were then averaged to provide a representative Raman spectrum of each sample. All spectra acquired were preprocessed in the OMNIC™ for Dispersive Raman software (version 9.8.372) using a sixth-order polynomial fluorescence correction prior to data analysis.

A strong linear correlation (*Average* R^2=0.98) was found to exist between COHb concentration and the ratio of the peak intensities at approximately 1,552cm^{-1} and 1,580cm^{-1}. From the eight calibration curves analyzed, the limits of detection and quantitation were found to be 4.4% and 13.2% COHb, respectively. Intra- and inter-day studies of three calibrators (10%, 30%, and 50% COHb) indicated high precision, with the coefficient of variation ranging from 0.85%–4.26%. The accuracy of this method was evaluated through the quantitation of ten experimental unknowns, each analyzed in triplicate. Using the proposed Raman microspectrophotometric method, the true concentrations of seven of the unknowns were within the 95% confidence limits of the predicted COHb concentrations, with the percent error ranging from 2.00%–54.00%. The highest error was associated with unknowns having a COHb concentration at or below 10% COHb, below the limit of quantitation. The unknowns were subsequently analyzed using a Cary 3500 UV-Visible spectrophotometer, resulting in only six of the unknowns' true concentrations being within the 95% confidence limits of the predicted COHb concentrations, with the percent error ranging from 6.86%–77.20%. In this case, the highest error was associated with an unknown that had been correctly quantitated via the Raman microspectrophotometric method with a percent error of 3.60%.

This study demonstrates that Raman microspectrophotometry may be used to quantitate the COHb concentration of blood samples. Furthermore, this method is comparable with established spectrophotometric methods while eliminating the need for time-consuming sample preparation and limiting the use of consumables and the generation of waste.

Carbon Monoxide, Carboxyhemoglobin (COHb), Raman Microspectrophotometry

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