Case Study:
"Detection of diluted controlled substances using a computational Raman spectroscopy technology (Rametrix®): Potential use in Point-Of-Care management of diversion"

Amr Sayed Issa, BSc Pharm, MS Biomedical Engineering, NABP, Research Engineer, DialySensors Inc., 1872 Pratt Drive Suite 1300, Blacksburg, VA 24060
E-mail: amrs@vt.edu

William Lee, DPh, MPA, FASCP, Director of Pharmacy Innovations, Carilion Clinic, 1906 Belleview Ave. SE, Roanoke, VA 24014
E-mail:wtlee@carilionclinic.org

Ryan Senger, PhD, Associate Professor, Biological Systems Engineering, Virginia Tech, HABB1, 1230 Washington St. SW, Blacksburg, VA, 24061,
Chief Technical Officer, DialySensors Inc., 1872 Pratt Drive Suite 1300, Blacksburg, VA, 24060,
E-mail: senger@vt.edu

Anita Kablinger MD, CPI, Professor and Director Clinical Research, Department of Psychiatry and Behavioral Medicine, Virginia Tech Carilion School of Medicine, 2017 S. Jefferson Street, 1st Floor Administrative Suite, Roanoke, VA 24014
E-mail: askablinger@carilionclinic.org

Robert Trestman, PhD MD, Professor and Chair, Department of Psychiatry and Behavioral Medicine, Virginia Tech Carilion School of Medicine, 2017 S. Jefferson Street, 1st Floor Administrative Suite, Roanoke, VA 24014
E-mail: trestman@vt.edu

John Robertson, VMD PhD, Research Professor, Biomedical Engineering and Mechanics, Virginia Tech, Kelly Hall, 325 Stanger St., Blacksburg, VA, 24061, President, DialySensors, Inc., 2000 Kraft Drive Suite 1208, Blacksburg, VA, 24060
E-mail: drbob@vt.edu

Abstract

Purpose

The diversion and loss of controlled substances (CS) through criminal adulteration and during 'wasting' procedures is a significant problem in hospitals, the entire healthcare industry, and society, in general. Dilution and replacement of CS with water, saline, or other liquids not only deprives patients of much-needed drugs but also may expose them to infectious agents.

Current practices to control diversion and loss are rarely targeted at the points of formulation, dispensing, use, and disposal (i.e., points-of-care [POCs]). There are few analytical systems available to rapidly screen liquid formulations of CS for dilution or adulteration at POCs; the operation of those systems currently in use is both labor-intensive and time-consuming.

We developed a Raman spectroscopy-based method for detecting dilution and/or adulteration of liquid CS.

Methods

We obtained reference Raman spectra for six commonly prescribed CS and then subsequently collected spectra from serially-diluted CS test articles.

Results

Our methods were able to detect dilution to 1-5% of the original test article concentration. Measurements required less than one minute, did not alter the composition of the samples, and could be reported against a reference database in real-time.

Conclusion

Efforts are currently underway to apply these quantitative Raman spectroscopy-based methods for automated screening up to 100% of CS at POCs.

Introduction/Background

Controlled substances (CS) are essential in every hospital for anesthesia, pain management, and other therapies[1]. CS distribution and use is highly regulated by the Drug Enforcement Administration (DEA) and state agencies to detect/correct misuse, substandard practices, or criminal diversion. Penalties by DEA and consequential enforcement actions by the Department of Justice for CS misuse/diversion are severe. A recent CS diversion incident in Virginia and North Carolina resulted in a $4.3 million fine to several regional healthcare systems and criminal prosecution/imprisonment of a staff member who stole CS from a Pyxis ® automated dispensing system[2]. According to pharmacy managers, millions of dollars are invested annually in CS inventory programs, dispensing and unit dosing systems, 'smart technology' (high-definition cameras/facial recognition) in pharmacies/storage sites, personnel behavioral education, drug diversion software, and oversight of staff administering CS[3].

Despite the many programs, technologies, and procedures in place, CS diversion is problematic and widespread. According to the Joint Commission, many hospitals do not meet or have lax, inefficient enforcement of medication security standards[4]. Injectable CS (e.g., morphine, fentanyl) were diluted/replaced/wasted/'lost' at many points of transit from pharmacies to, or at, high-risk Points-Of-Care (POCs), including emergency departments, endoscopy, oncology, obstetrics-gynecology, post-surgical recovery, and intensive care facilities)[5.] Diversion by staff (including doctors and nurses with addictions) has been recognized and well-documented. Aside from depriving patients of critically needed pain management drugs, inadvertent administration of adulterated, contaminated CS to hospitalized patients has resulted in deaths[6].

We analyzed common, current hospital procedures for preventing CS diversion and identified a critical point of diversion not addressed by current CS practices or technology. CS not used for patient care at the point of care (POC) are required, by law, to be inventoried (measurement of unused volume of drug) and 'wasted' (flushed in a toilet, for example) or destroyed by other secure means. Impartial witnessing of 'wasting' is required in most hospitals. However, unobserved 'skillful' adulteration (removal of CS, replacement with other fluids) has been performed before, or at, the time of POC disposal. Chemical analysis of CS at the POC is rarely done voluntarily or is operationally impractical (fewer than 5% of CS are routinely analyzed). Current methods (measuring specific gravity, for example) may not detect 'skillful' (criminal) adulteration. The testing and "wasting" of CS in the context/use by emergency medical services (EMS) is not even addressed in many healthcare systems and is poorly monitored at best.

The use of Raman spectroscopy for spot drug identification is well-known in hospital, pharmacy, and law enforcement venues. Current applications of the technology are focused on qualitative (i.e., What CS is present?) rather than quantitative analysis (i.e., What CS is present, how much of it is present, and has it been adulterated or replaced by dilution?). These commercially available Raman spectroscopy-based CS analysis systems usually compare the Raman spectrum of samples being tested ('test articles')

with reference spectra present in a generic database which may or may not be site-specific, lot-specific, formulation-specific, or readily updated for local healthcare settings.

Over the past five years, we have developed and validated a technology (Rametrix®), based on Raman spectroscopy-based chemometric analysis. Rametrix® has been used to determine the identities and quantities of molecules in complex aqueous solutions, such as parenteral CS solutions. Here, we report the results of a study in which we intentionally diluted ('adulterated') commonly used CS and then performed qualitative and quantitative analyses with our Rametrix® technology. We believe these results indicate this method could be easily applied to detection of drug diversion (including dilution) at POCs.

Materials and Methods

The purpose of this study was to identify the Raman spectral signatures of representative and commonly used CS: fentanyl, ketamine, methadone, midazolam, morphine sulfate, and propofol. From here, it was determined if (i) identification of the CS and (ii) dilution (adulteration) of these substances could be detected using a Raman spectroscopy and Rametrix® at the POC. The study was performed at the Carilion Clinic Pharmacy in Roanoke, VA USA and under the supervision of managing pharmacists.

Sample preparation and procedures. All CS test articles used in this study, manufacturers, lot numbers, and stock concentrations (millimolar - mM) are given in Table 1. Dilutions of each CS test article were prepared using 0.9% preservative-free saline (Hospira). The following concentrations, relative to stock (mM), were prepared: 100% (i.e., the stock concentration), 50%, 25%, 10%, and 1% (i.e., 1% stock, 99% saline). These dilutions (1.5 mL working volume each) were stored in 2 mL flat bottom clear borosilicate glass vials (0.78 mm glass thickness) (Thermo Fisher Scientific, Waltham, MA) for immediate analysis by Raman spectroscopy. Samples were scanned with Raman spectroscopy on-site (i.e., at the POC), and all samples (in analysis vials) were returned immediately for destruction. The return of samples and their destruction were recorded and witnessed in compliance with hospital CS procedures.

Table 1. CS test articles used in this study.

Raman spectroscopy. Bulk liquid samples were analyzed by two different Raman spectrometers: (i) a WP 785 semi-integrated spectrometer (Wasatch Photonics; Morrisville, NC USA) and (ii) an Agiltron PeakSeeker Pro (Agiltron; Woburn, MA USA). For both instruments, Raman scans were acquired with a 785 nm laser with 100 mW laser intensity, 0.2 mm laser spot size, 15 s exposure time, and 15 s delay between scans. Spectra were acquired over a 200-2,400 cm^{-1} range. Dark scans were collected and subtracted from all spectra. Data collected from the Agiltron instrument were processed with RSIQ software (Agiltron), and data were collected from the Wasatch Photonics instrument using Enlighten v.2.2.7 software (Wasatch Photonics).

Raman spectra processing and baselining. All computational processing of Raman spectra was performed using the publicly available Rametrix® LITE[7] and PRO[8] Toolboxes in MATLAB r2022a (MathWorks; Natick, MA). The acquired Raman spectra were averaged among 10 scan replicates and truncated to the fingerprint Raman shift range of 400-1,800 cm^{-1}. Next, spectra were baselined using ISREA[9] and vector normalized. ISREA nodes were chosen to preserve signature Raman bands of all CS test articles. Up to eight nodes were used along the Raman shift axis and were used to connect cubic splines of the ISREA algorithm to form spectral baselines.

Qualitative analysis. Processed and baselined spectra were then subjected to principal component analysis (PCA), multivariate analysis of variance (MANOVA), and pseudo quadratic discriminate analysis (DA). The procedure is referred to as discriminant analysis of principal components (DAPC) here. A DAPC model was constructed with the top five principal components (PCs) of all averaged, baselined, and processed spectra (all CS and concentrations) with the goal of identifying the CS identity of an unknown sample. The DAPC model was validated through leave-one-out cross-validation, and the overall prediction accuracy, sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV) were calculated based on the model predictions for left-out samples. The procedure has been demonstrated in prior studies[10-13].

Quantitative analysis. Concentrations for each CS were calculated from Raman spectra using PCA followed by partial least-squares regression (PLSR) with processed and averaged spectra as shown previously[14]. Here, the top four PCs were used in model construction, along with up to three PLSR components. The PCA-PLSR models were generated for each CS separately and used separate sets of ISREA nodes.

Results

Raw and baselined Raman spectra. Representative Raman spectra are shown for the CS trial articles of this study in **Fig. 1.** An example of a raw Raman scan of methadone, before scan replicate averaging, ISREA baselining, and vector normalization is given in **Fig. 1A.** The replicate averaged, ISREA baselined, and vector normalized spectra for all CS are given in **Fig. 1B.** These are considered the Raman spectral "fingerprints" of each CS test article. The ISREA nodes used to generate the baseline were located at 400, 561, 609, 890, 1224, 1498, 1732, 1800 cm^{-1}. Comparison of the methadone spectra in **Figs**. **1AB** demonstrate the spectral transformation accomplished by ISREA.

Figure 1. Representative Raman spectra of CS test articles analyzed in this study (fentanyl, ketamine, methadone, midazolam, morphine sulfate, and Propofol). (A) Raw (unprocessed) Raman spectrum of methadone and (B) Raman spectral fingerprints consisting of: replicate averaged, ISREA baselined, and vector normalized spectra. Stock concentrations of all CS test articles are represented in the figure.

Qualitative analysis. DAPC models were built in effort to identify an unknown CS given only its Raman scan. All replicated averaged, ISREA baselined, and vector normalized spectra were used in model construction. The same ISREA nodes used in **Fig. 1** were used in this analysis. Approximately 98% of the dataset variance were represented by the top five PCs used in DAPC model construction. Leave-one-out analysis was applied to cross-validate the model. The prediction accuracy, sensitivity, specificity, PPV, and NPV for all left-out samples were calculated and are referred to as "prediction metrics". With the set of ISREA nodes mentioned above, all prediction metrics for all CS test articles were near 100%, as shown in **Table 2.** The only incorrect prediction was for the 1% of stock concentration of Propofol (the largest dilution), which was predicted to be fentanyl.

Table 2. Prediction metrics for identifying CS test articles.

The PCA and DAPC cluster plots are shown in **Fig. 2**. The DAPC cluster plot is a result of MANOVA. This process is also referred to as fingerprinting analysis and illustrates differences in spectra resolved by statistical analysis. In both plots (**Fig. 2**), each data point represents a processed (and replicate averaged) CS test article Raman spectrum, and clustering is indicative of fingerprint similarity. Clustering was observed in both PCA and DAPC analyses. Separation by concentration was also observed in PCA (**Fig. 2A**). Regions of low concentration are highlighted in both analyses by the dashed circle. The DAPC analysis was able to resolve 11/12 samples diluted to 10% and 1% of stock concentration. Several fall within this low concentration region. It is noted that the stock concentration of fentanyl (0.05 mg/mL, **Table 1**) falls outside of this low concentration region.

Figure 2. Cluster analysis based on (A) PCA and (B) MANOVA results of the DAPC model. This also referred to as fingerprinting. Individual data points represent processed Raman spectra for each CS test article.

Quantitative analysis. PLSR analysis was applied to each CS test article, separately, to determine the accuracy at which concentrations (dilutions) could be identified by Raman spectroscopy and Rametrix® computations. Our methods were able to detect dilution to 1-5% of the original test article concentration. An example of one such analysis is shown in **Fig. 3**, where it was found that the correlation between the concentration of Methadone and its processed Raman spectra was R^2 = 0.993 (**Fig. 3D**). The R^2 values for all CS test articles are given in **Table 2**. For each CS test article, it was found that separate sets of ISREA nodes were required to achieve high R^2 correlations. These are also given in **Table 3**.

Figure 3. PLSR results showing the correlation of processed Raman spectra with methadone dilutions. (A) model training to show the percent variance explained by PLS components, (B) the model training set (when all samples are included in model construction), (C) Prediction error for leave-one-out analysis, and (D) the comparison of actual concentration (% of stock) to predicted concentration (% of stock) from Raman spectra and Rametrix® during leave-one-out trials.

Table 3. Correlations of CS test article dilutions with processed Raman spectra.

Discussion

This study showed that six CS test articles could be detected and differentiated from one another in aqueous solutions at concentrations ranging from 1% to 100%. Their concentrations could also be deduced from their Raman spectra. These measurements were made at the POC using benchtop Raman spectrometers and a laptop computer. The analysis of individual samples took less than five minutes, and no sample preparation (other than transferring to a glass vial) or chemical derivatization was required. Based on the results obtained in this study, the CS identity was predicted correctly in all cases, down to 10% of stock concentration. At 1% of stock concentration, 5/6 samples were identified correctly. However, in the case of drug dilution/diversion with saline, the analysis proved capable of detecting reduction in CS concentration and identifying where drugs may fall into a low concentration category.

In the study, all dilutions were performed in a laminar flow hood with saline 0.9%, preservative-free USP, minimizing introduction of contaminants that could be detected with other diluents, including non-sterile mixtures. This is a limitation of the current approach, as in drug diversion/dilution cases, many solvents could be used as a diluting agent. For example, intentional adulteration of CS with fluids such as tap water, or other parenteral fluids commonly used in hospitals, is very likely to not only to dilute the parent CS but also introduce other molecules (bacterial byproducts or water treatments, for example). Thus, additional studies are needed to include more CS test articles and diluting agents in our Raman spectral database, which now contains the six CS test articles with saline dilutant. Several diluting agents have been used in drug diversion case studies, and it will be necessary to complete construction of a Raman spectral database considering several/all of these. It is intended to keep these unpublished for now for law enforcement purposes. However, the analysis presented here can also be used as a "yes/no" screen to (i) validate the identity of CS test article, (ii) determine whether or not it is at stock concentration, and (iii) probe for any additional contaminants/dilutants in the sample.

In summary, the described Raman spectroscopy and Rametrix®-based technology could be useful at POCs in hospitals to detect adulteration and diversion of CS quickly and inexpensively. While the study here is a proof-of-concept, it establishes a framework for building a large Raman spectral database that could serve to scan CS fluids at POCs to ensure compliance and detect/prevent CS dilution/diversion.

References

1. Yaksh, T, Wallace, M. Chapter 20: Opioids, analgesia, and pain management. *In* Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e. Brunton LL, Hilal-Dandan R, Knollmann BC. eds. McGraw Hill; 2018. pp 356-386.

2. Nurse sentenced to 54 month for drug tampering. [https://www.justice.gov/usao-mdnc/pr/nurse-sentenced-54-months-drug-tampering] (accessed 2022 June 23).

3. Safety topics A-Z. Opioid and drug diversion. [https://www.premiersafetyinstitute.org/safety-topics-az/opiods/drug-diversion/] (accessed 2022 June 23).

4. Drug diversion and impaired healthcare workers. [https://www.jointcommission.org/resources/news-and-multimedia/newsletters/newsletters/quick-safety/quick-safety-48-drug-diversion-and-impaired-health-care-workers/#.YrIRwHbMKUk. (accessed 2022 June 23).

5. Nolan K, Zullo AR, Bosco E, Marchese C, Berard-Collins C. Controlled substance diversion in health systems: A failure modes and effects analysis for prevention. *Am J Health Syst Pharm*. 2019 Jul 18;76(15):1158-1164. doi: 10.1093/ajhp/zxz116. PMID: 31408513; PMCID: PMC7170724.

6. Breve F, LeQuang J K, Batastini L. Controlled substance waste: concerns, controversies, solutions. *Cureus* 14(2): e22564.DOI 10.7759/cureus.22564.

7. Fisher, AK, Carswell, WF, Athamneh, AIM, et al. The Rametrix® LITE Toolbox v1.0 for Matlab. *J Raman Spectrosc* 2018. 49(5): 885-896. doi:10.1002/jrs.5348.

8. Senger, RS, Robertson, JL. The Rametrix® PRO Toolbox v1.0 for Matlab. *PeerJ* 2020. 8: e8179. doi:10.7717/peerj.8179.

9. Xu, Y, Du, P, Senger, RS, et al. ISREA: An efficient peak-preserving baseline correction algorithm for Raman Spectra. *Appl. Spectrosc* 2021. 75(1): 34-45. doi:10.1177/0003702820955245.

10. Senger, RS, Sullivan, M, Gouldin, A, et al. Spectral characteristics of urine from patients with end-stage kidney disease analyzed using Raman chemometric urinalysis (Rametrix®). *PLoS One*. 2020. 15(1): e0227281. doi:10.1371/journal.pone.0227281.

11. Senger, RS, Scherr, D. Resolving complex phenotypes with Raman spectroscopy and chemometrics. *Curr. Opin. Bio- technol*. 2020. 66: 277-282. doi:10.1016/j.copbio.2020.09.007

12. Huttanus, HM, Vu, T, Guruli, G, et al. Raman chemometric urinalysis (Rametrix®) as a screen for bladder cancer. *PLoS One.* 2020. 15(8): e0237070. doi:10.1371/journal.pone.0237070.

13. Senger RS, Sayed Issa A, Agnor B, et. al. Disease-associated multimolecular signature in the urine of patients with Lyme disease detected using Raman spectroscopy and chemometrics. *Appl Spectrosc.* 2022 Mar;76(3):284-299. doi: 10.1177/00037028211061769. Epub 2022 Feb 1. PMID: 35102746.

14. Carswell W, Robertson JL, Senger RS. Raman spectroscopic detection and quantification of macro- and microhematuria in human urine. *Appl Spectrosc.* 2022 Mar;76(3):273-283. doi: 10.1177/00037028211060853. Epub 2022 Feb 1. PMID: 35102755.

Tables and Figures

Table 1. CS test articles used in this study.

Brand/ Generic	NDC	Form	Strength (mg/mL)	Manufacturer	Lot # 1	Lot # 2	Lot # 3	Chemical Name
Morphine Sulphates	0641612701	Injectable solution, USP IV only	10 mg/mL	Hikma	40134	70108	40125	Morphine Sulphate
Fentanyl	0641602701	Fentanyl Citrate INJ. USP IM/IV	0.05mg/mL	West-Ward	60002	101007	NA	Fentanyl
Midazolam	0641620901	HCl injection,USP IM/IV	1mg/mL	West-Ward	510077	NA	NA	Midazolam
Ketamine	0143950801	HCl injection,USP IM/IV	50 mg/mL	Hikma	2105183-1	NA	NA	Ketamine
Diprivan	6332326929	Injectable Emulsion, USP	10mg/mL	Fresenius Kabi	10QE6563	NA	NA	Propofol
Methadone	0054355563	HCL Oral Solution	1mg/mL	Hikma	21332889	NA	NA	Methadone
Sodium Chloride PFS	0409488803	0.9% Saline Solution	0.90%	Hospira	FK8409	FK8409	FK8409	Saline

Table 2. Prediction metrics for identifying CS test articles.

CS Test Article	Overall Accuracy*	Sensitivity	Specificity	PPV	NPV
Fentanyl	97%	100%	96%	83%	100%
Ketamine	100%	100%	100%	100%	100%
Methadone	100%	100%	100%	100%	100%
Midazolam	100%	100%	100%	100%	100%
Morphine Sulfate	100%	100%	100%	100%	100%
Propofol	97%	80%	100%	100%	96%

* 29/30 samples were predicted correctly

Table 3. Correlations of CS test articles dilutions with processed Raman spectra.

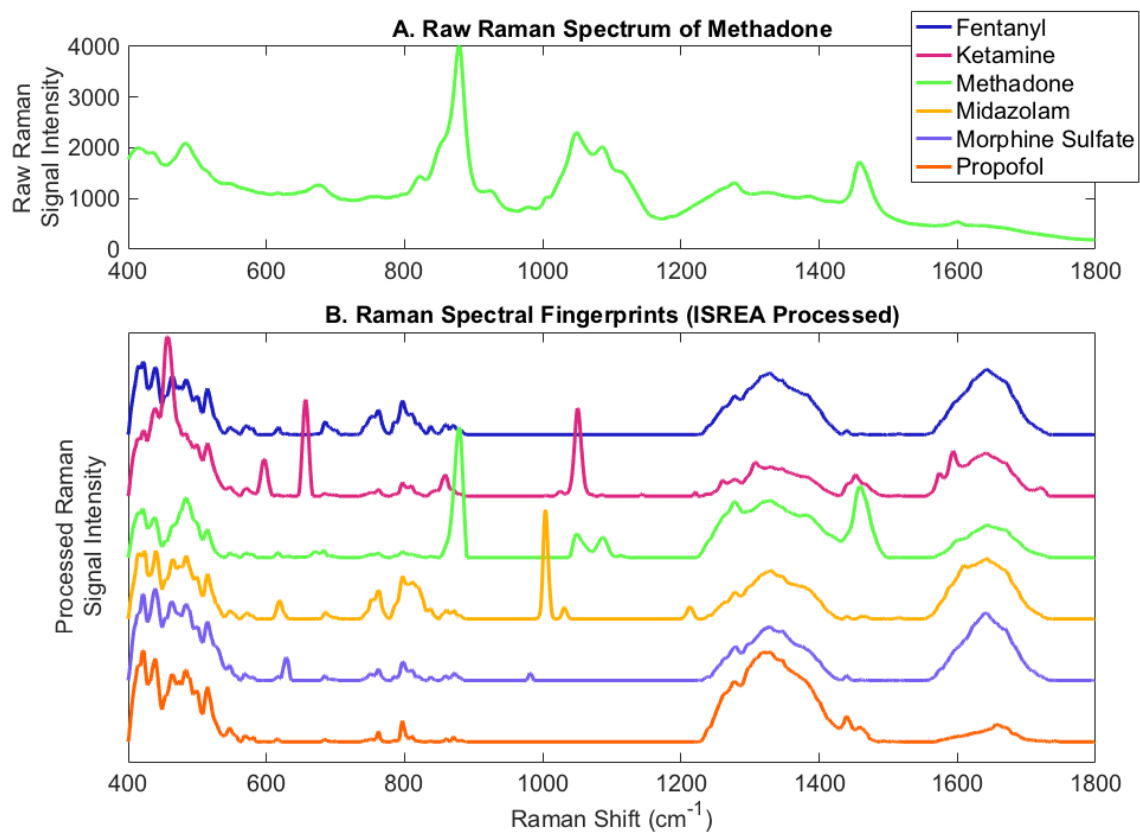| CS Test Article	ISREA Nodes (cm^{-1})	R^2
Fentanyl	400, 588, 688, 697, 984, 1128, 1795, 1800	0.989
Ketamine	400, 419, 497, 1093, 1128, 1223, 1764, 1800	0.996
Methadone	400, 1169, 1397, 1441, 1523, 1538, 1787, 1800	0.993
Midazolam	400, 653, 800, 1000, 1362, 1400, 1600, 1800	0.999
Morphine Sulfate	400, 405, 466, 1060, 1110, 1395, 1657, 1800	0.999
Propofol	400, 770, 887, 1174, 1491, 1617, 1678, 1800	0.991

Figure 1. Representative Raman spectra of CS test articles analyzed in this study (fentanyl, ketamine, methadone, midazolam, morphine sulfate, and Propofol). (A) Raw (unprocessed) Raman spectrum of methadone and (B) Raman spectral fingerprints consisting of: replicate averaged, ISREA baselined, and vector normalized spectra. Stock concentrations of all CS test articles are represented in the figure.
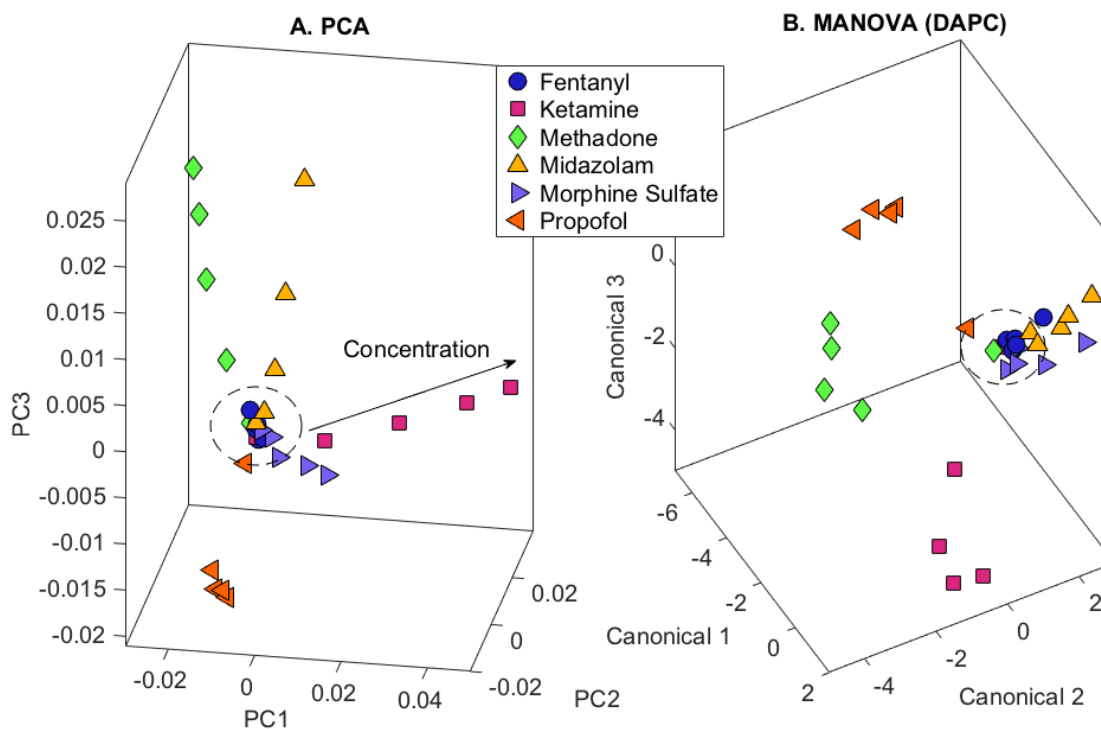
Figure 2. Cluster analysis based on (A) PCA and (B) MANOVA results of the DAPC model. This also referred to as fingerprinting. Individual data points represent processed Raman spectra for each CS test article.
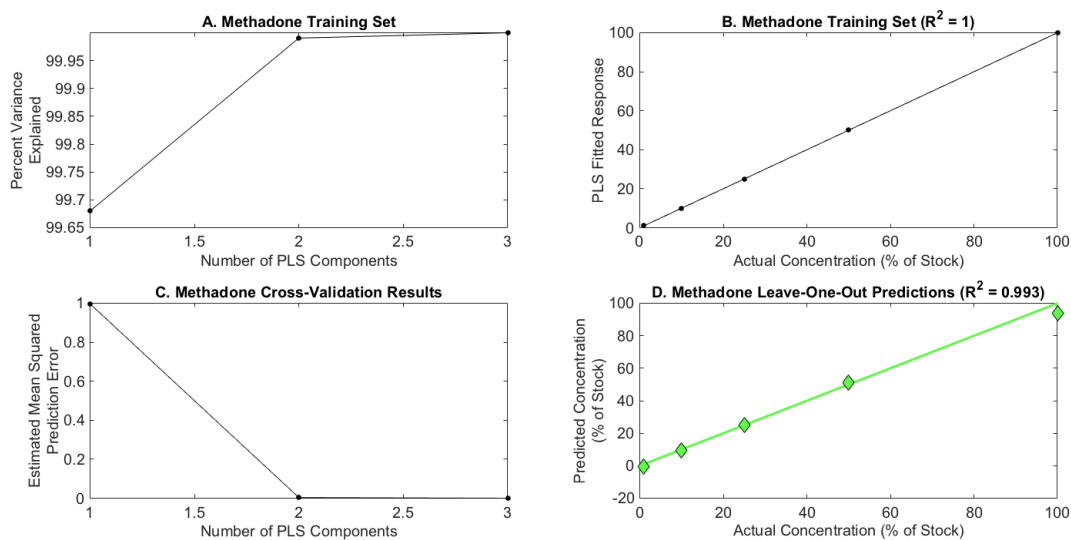
Figure 3. PLSR results showing the correlation of processed Raman spectra with methadone dilutions. (A) model training to show the percent variance explained by PLS components, (B) the model training set (when all samples are included in model construction), (C) Prediction error for leave-one-out analysis, and (D) the comparison of actual concentration (% of stock) to predicted concentration (% of stock) from Raman spectra and Rametrix® during leave-one-out trials.