BRITISH COLUMBIA CENTRE ON SUBSTANCE USE

Networking researchers, educators & care providers

Drug Checking

An Assessment of Two Point-of-Care Fentanyl Quantification Methods Using Fourier-transform Infrared Spectroscopy

February 2021

Land Acknowledgement

The BC Centre on Substance Use would like to respectfully acknowledge that the land on which we work is the unceded ancestral homelands of the xwmekwey'em (Musqueam), Skwxwú7mesh (Squamish), and sel'ílweta| (Tsleil-Waututh) Nations.

Authors

Samuel Tobias, MSc
Senior Drug Checking Operational Technician, British Columbia Centre on Substance Use

Lianping Ti, PhD
Research Scientist, British Columbia Centre on Substance Use;
Assistant Professor, Department of Medicine, University of British Columbia

Funding

This report was supported by a Health Canada Substance Use and Addictions Program grant to the BC Centre on Substance Use to implement and evaluate a drug checking pilot in British Columbia (Arrangement #: 1718-HQ-000024). Funding agencies had no role in the research, design, or writing of the report, nor did they have a role in the decision to publish it. Findings reported here should in no way be taken as an endorsement of the specific point-of-care technologies that were used for this study, and the authors declare no conflicts of interest.

Acknowledgments

The authors would like to thank the staff at Health Canada's Drug Analysis Service for generously providing confirmatory testing services, particularly Richard Laing, Francine Chartier, and Lilly Luu. They would also like to thank Hadley Pearce for her assistance with editorial design.

Contact

Learn more about www.drugcheckingbc.ca
For further drug checking-related inquiries, you can reach us at drugchecking@bccsu.ubc.ca

Summary

In British Columbia, drug checking with Fourier-transform infrared (FTIR) spectroscopy allows for two different quasi-quantitative methods for determining fentanyl concentration: mixture analysis and Bruker Quantitative Analysis 2 (QUANT 2). Neither of these methods have been formally evaluated and validated for this specific purpose. We assessed these two methods' ability to estimate fentanyl concentration in drug checking samples by comparing the results to a laboratory reference standard, quantitative nuclear magnetic resonance (qNMR). Our findings indicate that the mean absolute differences in concentration comparing qNMR with mixture analysis and QUANT 2 was 5.2% and 1.1%, respectively, with both methods showing a tendency to underestimate fentanyl concentrations at higher concentrations and overestimate fentanyl concentrations at lower concentrations. Further method developments are needed for these two techniques, given that there are potential harms associated with providing individuals inaccurate quantification information. To better quantify fentanyl at point-of-care, alternatives to using the Bruker software or mixture analysis may allow for further refinement and assessment.

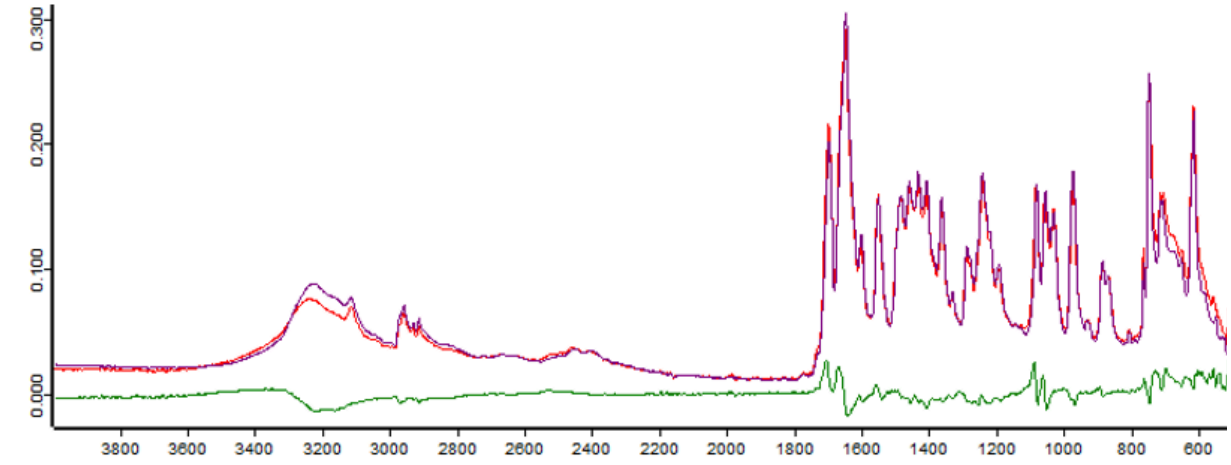
.

Background

In response to the unprecedented rise in overdose deaths in recent years, harm reduction organizations that offer drug checking have turned to Fourier-transform infrared (FTIR) spectroscopy as the method of choice as it offers many benefits over more costly, time-consuming, or destructive methods.[1–4] Briefly, drug checking using FTIR works by scanning a drug sample on a spectrometer to produce a spectrum, a visual representation of the absorbance of infrared light by the sample.[5] The spectrum is then searched against a set of reference libraries to determine matches and identify components, as compounds have unique and differentiable spectra. Although not an inherently quantitative method (i.e., allows for absolute quantification of a compound by weight), FTIR offers methods to quasi-quantify desired components relative to others. In British Columbia (BC), a large number of drug checking services utilize Bruker ALPHA or ALPHA II FTIR spectrometers and corresponding OPUS Software (Billerica, MA, USA) which offers two ways to report quantification results: 1) mixture analysis and 2) Bruker Quantitative Analysis 2 (QUANT 2).

The first, mixture analysis, is an algorithm that attempts to recreate the query spectrum (drug sample) using library entries to best represent it, and therefore can report the ratio of components as a percentage make-up (Figure 1).[6] The second, QUANT 2, is a validated prediction model that allows for multivariable calibrated analysis, and similarly, is designed to quantitatively analyze spectra to determine component concentrations.[7] To do this, QUANT 2 utilizes a partial least squares fit method which correlates spectral intensity in specified wavelength regions with values that were generated from reference entries (i.e. point-of-care FTIR scans later quantified by confirmatory methods).

Until now, drug checking in BC has relied mostly on mixture analysis to determine fentanyl concentration of drug samples for two main reasons: accessibility and cost. QUANT 2 requires confirmed quantification information to build and validate a model, and there are additional costs associated with acquiring QUANT 2 as it is not a base functionality of OPUS. Neither of these methods have been formally evaluated and validated with point-of-care drug checking samples. This report further details these two methods, their advantages and disadvantages, and considers the future developments of point-of-care quantification of fentanyl in BC.



	Color	Content [%]	Compound Name	CAS number	Molecular formula
1		56.8	Caffeine		
2		28.4	Erythritol		
3		14.8	Fentanyl HCl		

Figure 1. Example of a successful mixture analysis output of a sample. The red line is the measured sample and the purple line is a composite of the estimated percentages. The green line is the residual (difference between the two).

Methods

The data were derived from a dataset of over 1,000 confirmed drug checking samples in BC between April, 2018 and May, 2020. In total, 55 samples were selected to be analyzed using the two point-of-care quantification methods. The basis for the selection of these specific samples were that they were confirmed to contain fentanyl hydrochloride (as opposed to other fentanyl analogues), and best resemble typical illicit opioids (colloquially referred to as 'down' in BC) and therefore, they are likely to comprise representative samples that come across any drug checking shift. The samples were analyzed using the developed QUANT 2 model, and then mixture analysis. Mixture analyses were conducted using a single reference library (BCCSU FTIR-ATR Library of Drugs and Common Adulterants) and were repeated with increasing component parameters until a fentanyl value was obtained.

First, we described the different components found in the included fentanyl drug samples. Second, we assessed the difference in fentanyl concentrations among samples that mixture analysis failed to return fentanyl quantification results for compared to those that mixture analysis successfully returned fentanyl quantification results for. Failure, in this case, is defined as the inability to obtain a mixture analysis output containing a fentanyl quantification value when fentanyl was identified during the primary subtractive analysis. Lastly, results from the two point-of-care methods were plotted against the respective qNMR result (i.e., true value), and differences and a coefficient of determination (R2) for each method were calculated.

Results

Other than fentanyl, FTIR analysis indicated the 55 drug samples contained various other components: caffeine, sugar alcohols (mannitol, inositol, erythritol, xylitol), heroin, polyethylene glycol, and Vitamin C. Five samples where the qNMR results were over 20% were considered outliers and were removed as the inclusion of these samples would drastically skew results. Overall, fentanyl concentrations (as determined by qNMR) for the remaining 50 samples ranged from 2.1 to 18.0 %. Of the 50 samples analyzed, mixture analysis failed to return fentanyl quantification results for 17 (34.0%) samples. Mixture analysis failed to return results in samples confirmed to contain fentanyl ranging from 2.1% to 15.0% (mean 7.6%) (Figure 2).

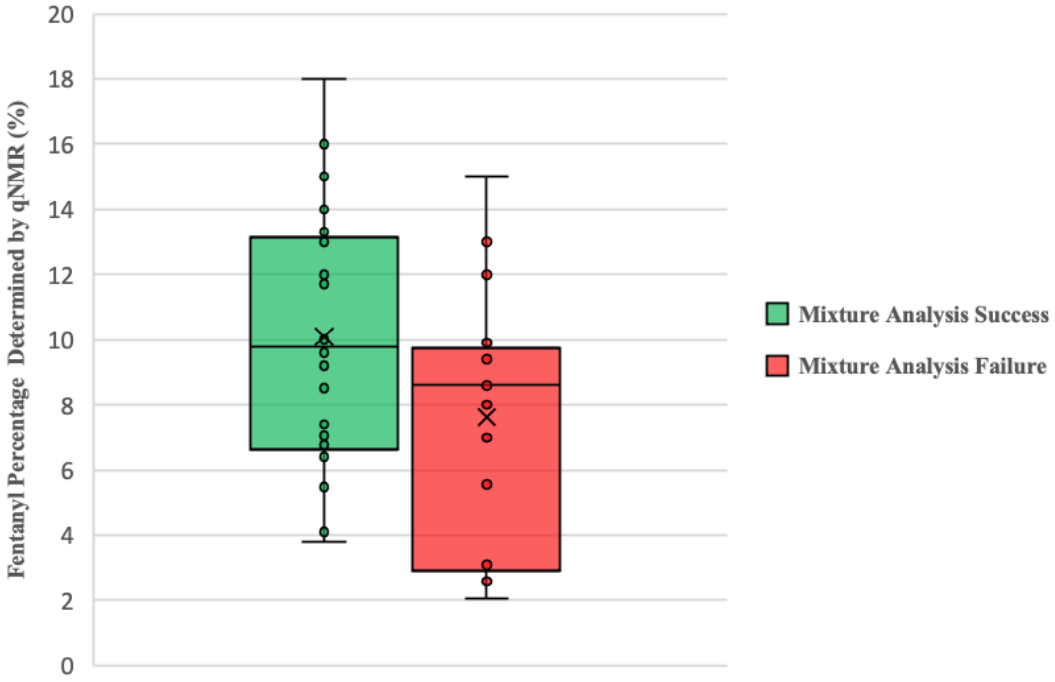


Figure 2. Box and whisker plots indicating descriptive statistics for fentanyl samples that returned mixture analysis results (green) and failed to return results (red).

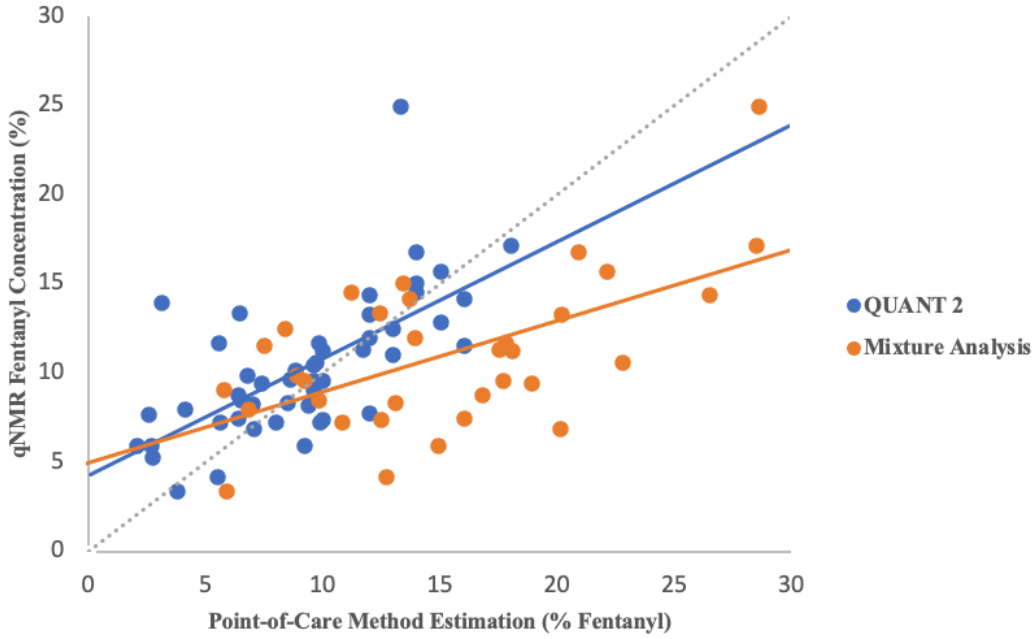


Figure 3. Scatter plot of the point-of-care fentanyl estimation by QUANT 2 (blue, n=50) and mixture analysis (orange, n=33) with regression lines. The grey dotted line indicates perfect correlation.

Descriptive statistics of differences from the true value (qNMR fentanyl concentration) for mixture analysis indicated a mean of -5.2, median of -5.8, and a range of -15.3 to 8.5. For QUANT 2, the differences from qNMR had a mean of -1.1, median of -0.9, and a range of -11.7 to 4.4. The positive correlation of values was stronger for QUANT 2 and qNMR ($R2 = 0.45$) compared to mixture analysis and qNMR ($R2 = 0.34$) (Figure 3). At low concentrations, both QUANT 2 and mixture analysis tend to overestimate fentanyl concentrations, and at high concentrations, they tend to underestimate.

Summary of Findings

Mixture analysis is a proprietary algorithmic function in OPUS that attempts to recreate the query spectrum (measured sample) using library entries. The output (example in Figure 1) returns estimated concentrations of components in a percentage format. When performing a mixture analysis, there are two factors that need to be selected: 1) the maximum number of components to return and 2) which libraries to use. Running the same spectrum through mixture analysis but adjusting these factors will return different results. For drug checking, technicians are taught to determine which components are present first by using a method called subtractive analysis. When the components are identified, the total number of components can then be used as the parameter for the mixture analysis. A major issue with mixture analysis however, is that if an identified compound is 'missed' by the algorithm, it will not be returned as a result and because results always sum to 100%, the percentage of other compounds will be overestimated. Alternatively, if too high of a number of components is inputted, mixture analysis can return false results (i.e., the algorithm incorrectly identifies components and assigns them as percentage). To address this, drug checking technicians are taught to reject the presence of components returned by mixture analysis if they were not identified during the primary subtractive analysis.

In the present analysis, 34% of analyzed samples did not return mixture analysis results. That is, even by subjectively altering the input parameters of mixture analysis, there was no combination that returned a fentanyl concentration value. Drug checking technicians in BC have been under the assumption that this failure of mixture analysis to return fentanyl results occurs when fentanyl levels are low and are therefore 'missed.' However, Figure 2 indicates that this may be one of several other potential reasons, as mixture analysis successfully returned fentanyl results in samples within a similar range. It may be due to interfering signal of other components or samples having too many overlapping infrared bands.

It is important to provide individuals who access drug checking with accurate and consistent quantification information, particularly when the drug in question is potent like fentanyl. With mixture analysis, results are presented with a composite and residual spectrum where inferences about the results (i.e., whether they are over or underestimated) can be made. While QUANT 2 results are presented without contextual data, analysis of differences of QUANT 2 and mixture analysis from their corresponding confirmatory qNMR quantification reveals mean absolute differences of 1.1% and 5.2%, respectively, with a tendency to underestimate fentanyl concentrations at higher concentrations and overestimate fentanyl concentrations at lower concentrations. These findings suggest that there are limitations to both quantification methods, and both should be used with caution; however, QUANT 2 may be a more accurate model to implement in practice. It is noteworthy that these findings should not be extrapolated to samples where fentanyl concentrations are high (i.e., above 20%).

Conclusion

Further evaluation of the existing methods to quantify fentanyl at point-of-care is needed given the findings of this assessment. There are potential harms associated with providing individuals inaccurate quantification information and providing wide ranges to account for inaccuracies offers limited utilizable information to people who use drugs who access drug checking. To better quantify fentanyl at point-of-care, alternatives to using the Bruker software or mixture analysis may allow for further refinement and assessment.

References

1. Laing MK, Tupper KW, Fairbairn N. Drug checking as a potential strategic overdose response in the fentanyl era. Int J Drug Policy. 2018;62:59-66. doi:10.1016/j.drugpo.2018.10.001
2. Tupper KW, McCrae K, Garber I, Lysyshyn M, Wood E. Initial results of a drug checking pilot program to detect fentanyl adulteration in a Canadian setting. Drug Alcohol Depend. 2018;190:242-245. doi:10.1016/j.drugalcdep.2018.06.020
3. McCrae K, Tobias S, Grant C, et al. Assessing the limit of detection of Fourier-transform infrared spectroscopy and immunoassay strips for fentanyl in a real-world setting. Drug Alcohol Rev. 2020;39(1):98-102. doi:10.1111/dar.13004
4. Green TC, Park JN, Gilbert M, et al. An assessment of the limits of detection, sensitivity and specificity of three devices for public health-based drug checking of fentanyl in street-acquired samples. Int J Drug Policy. 2020;77:102661. doi:10.1016/j.drugpo.2020.102661
5. Tobias S, Shapiro AM, Grant CJ, Patel P, Lysyshyn M, Ti L. Drug checking identifies counterfeit alprazolam tablets. Drug Alcohol Depend. 2021;218:108300. doi:10.1016/j.drugalcdep.2020.108300
6. Bruker Corporation. OPUS/SEARCH User Manual. Version 7. March 10, 2017.
7. Bruker Corporation. OPUS Spectroscopy Software User Manual: QUANT. 2006.