Review Article
Open Access

A review of the use of alcohol congeners in the evaluation of alcohol facilitated sexual crimes

Abstract

Investigations of crimes that have been facilitated by using a drug(s) requires a thorough characterization of properly collected evidence and an informed interpretation of the results. Alcoholic beverages remain the main contributor to drug facilitated crimes (DFC), including drug-facilitated sexual assaults. As one of the most common beverages in the United States, it is important to be aware of all aspects of the chemical properties of alcoholic beverages and metabolites to determine if there is anything that can be used to assist in the criminal investigation. Extensive research has been conducted in the areas of Phase II alcohol metabolism biomarkers, but other biomarkers such as alcohol congeners have not come to the forefront of forensic alcohol analysis. Investigation of alcohol congeners may provide additional links needed in DFC cases. Currently, alcohol analysis is performed to determine a blood alcohol content (BAC) using Headspace Sampling-Gas Chromatography Coupled with Flame Ionization Detection (HS-GC-FID) to separate and detect the presence of specific concentrations of methanol, ethanol, acetone and isopropanol. This protocol remains the mainstay for alcohol analysis, but may be less effective in cases where an "after drinking" scenario is presented. (For example, in situations where drinking occurs after the incident or when one alcoholic beverage was added to another, resulting in an increase of the ethanol intake. HS-GC-FID is typically a validated method only for these four alcohols, which would not be able to detect consumption of different alcoholic beverages. At the present time, alcohol analysis does not allow for the determination of the specific alcoholic beverage consumed. Current purge and trap technology, which is available for GC-FID as well as the availability of on-line sampling methods has created the possibility to catalog congeners present in various beverages, as well as to determine congener concentrations in human blood or other matrices.

The use of alcohol congener analysis may allow for additional evidence provided in criminal investigations by the identification of different alcoholic beverages consumed. This review examines how drug facilitated crime is currently investigated, how alcohol congener analysis is currently used in driving under the influence cases, and finally how using new and classical methods of alcohol congener analysis will benefit drug facilitated crime investigations. This review allows for the reader to determine if the use of alcohol congener analysis can benefit a drug facilitated crime.

Keywords: alcohol congener analysis, congeners, ethyl alcohol, fermentation by-products, ingredient biomarkers

Volume 5 Issue 1 - 2019

Ashraf Mozayani, Melinda Wilson Hohler, Jasmine Drake
Department of Adminstration of Justice, Texas Southern University, USA

Correspondence: Ashraf Mozayani, Department of Adminstration of Justice, School of Public Affairs, Texas Southern University, Houston, Texas 77004, USA,
Email Ashraf.Mozayani@tsu.edu

Received: October 29, 2018 | **Published:** January 10, 2019

Introduction

Drug facilitated crime (DFC) is defined as an incident where the victim is incapacitated due to drug use or alcohol consumption. Previous research has divided DFC and specifically drug facilitated sexual assault (DFSA) into two categories: proactive and opportunistic. Proactive DFC is where the victim is either covertly or with force administered an incapacitating or disinhibiting (*.ie.* unable to give consent) substance by the perpetrator for the purpose of committing a crime. Opportunistic DFC is where a criminal engages in a crime with a victim who is intoxicated by his or her own actions, to the point of near or actual unconsciousness.[1] Forensic alcohol analysis is typically a mandated procedure after a forensic toxicology laboratory receives a kit with DFC evidence.[2] In the United States, this routinely involves the detection and quantification of concentrations of methanol, ethanol, isopropanol, and acetone in biological samples. This analytical protocol provides a blood alcohol concentration (BAC), which is universally used in various legal proceedings but provides no information as to the specific alcoholic beverage consumed. Obtaining this information would provide useful information for DFC investigations, including opportunistic DFC by being able to corroborate witness testimony of which beverage(s) were consumed, and proactive DFC by being able to determine if "drink spiking" or the addition of one alcoholic beverage to another alcoholic beverage led to increased ethanol intake.

Previous research has shown that the most common drug detected in DFC investigations is ethanol.[1] Alcohol is commonly abused, with the worldwide per capita consumption averaging 62.5 liters of pure ethanol per year.[3] Given the prevalence of alcohol and its contributions to vehicle accidents, fatal events and DFC, methods for determining blood alcohol concentration (BAC) and studies of alcohol metabolism were first published by Widmark in the 1920s and 1930s.[4–6] The method of headspace sampling with analysis by gas chromatography coupled with flame ionization detection (GC-FID) remains the gold standard today and was developed in the 1960s. Machata was responsible for some of the earliest studies of alcohol congeners and their subsequent appearance in blood.[7–10] Alcohol

© 2019 Mozayani et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and build upon your work non-commercially.

Copyright: ©2019 Mozayani et al.

congeners are compounds that contribute to the flavor and unique aromas of the particular alcoholic beverage in which they are found. These congeners are often produced during fermentation along with ethanol, or during aging or processing through the degradation of the beverage's organic components. This also includes solvents used in fermentation and distillation such as methanol, ethyl acetate, and even chemicals such as benzene and ethyl carbamate. Some investigators believe that congeners may contribute to a beverage's intoxicating effects.[11] Determining these compounds requires biomarker and metabolism information as well as information associated with establishing a validated, consistent sampling as well as instrumental method. Once purge and trap technology became available for the GC-FID, it was possible to catalog congeners that were present in blood using a modified Widmark formula.[12–17] It is this ability that may be able to add additional information to be able to name specific beverages consumed in opportunistic DFC investigations. Cataloging congeners present in biological samples may also be able to determine "drink spiking" in proactive DFC. As alcoholic beverages advance with broader flavor palettes, congener analysis would not prove as successful if profiles were not established. The determination of which congeners are present in a biological sample, the nature of the metabolites that are formed, and the proportions of each may also provide additional tools to an investigator to allow them to identify the type or specific beverage consumed. This identification has been used previously in Europe to challenge the "hip-flask" defense, and these challenges can be used to assist in the determination of which beverage was consumed in opportunistic DFC. This review will attempt to show the importance of alcohol congener analysis as it relates to assistance in drug facilitated crime (DFC) investigations.[18]

While there are current methods using the Widmark formula and modified Widmark formulas to back calculate and estimate the BAC at the time of incident, and while this method is accepted in many legal proceedings[12], there remains wide variations in individual metabolism and elimination rates unless a larger subject pool and more anthropomorphic variables are included in the back extrapolation[19–24] Alcohol analysis has not developed to a stage where body tissues may be analyzed in such a way that the determination of the precise source of consumed alcohol can be established. Using precise estimations of alcohol congener concentrations at the time of incident may allow for corroboration of witness testimony, or the determination that a higher alcohol concentration drink may have been added to a lower strength drink.[25] In addition, the half-life of ethanol and many congeners present may allow for the detection of unique identifiers outside the short time frame of detection of many of the commonly cited drugs like Gamma hydroxybutyric acid (GHB). Also, in DFC cases, it is often more dangerous to excessively consume alcohol than it is to be intoxicated by a psychoactive drug, because perpetrators are often encouraging alcohol use because the victim is less likely to resist advances.[25] It has been stated previously that congeners allow for conclusions to be made on the class of beverage consumed. The additional analysis of congeners specific to beverages may allow for additional information needed to identify specific beverages.[18] This review strives to investigate the current uses of alcohol congener analysis and how these current uses can be expanded to assist DFC investigations. The review was conducted by searching through the literature using keywords such as "alcohol congeners", "drug facilitated crime", "drug facilitated sexual assault" and "alcohol analysis". This search was also expanded adding combinations of the keywords that were used such as "Drug facilitated crime and alcohol"; and as the review continued "fermented by-products" and "ingredient biomarkers" were added.

Alcohol congeners

Fermented beverages have been found to contain greater than 800 congeners and more than 45 alcohols.[26–29] Concentration ranges are dependent on the beverage and can range from approximately 0.01mg/L to approximately 600 mg/L. Previous research about congeners has often concluded that congeners and their metabolites have an impact on the absorption of ethanol through the intestinal mucosa.[18] Various congeners are divided into two separate groups, fermentation by-products and ingredient a period after "biomarker products, alcohol congeners provide an insight into the class and specific beverage that was consumed. Fermentation by-products are formed during the fermentation or aging process or are added during processing. Ingredient biomarker products are congeners that are found as a result of the ingredients and materials used during production.[25] There are also members that cannot be distinguished into either group, making the labels not exclusive. Initially, limitations of the sensitivity using HS-GC-FID prevented congeners from being detected successfully. While HS-GC-FID still has limitations in sensitivity, sample preparation improvements including SPME allows for lower detection limits. This allows the typical instrument to reach the limit of detection for methanol down to approximately 0.1mg/L and down to approximately 0.01mg/L for other alcohol congeners. Also, this method allows for the separation and detection of the entire volatile profile including the classic alcohols commonly tested for in blood and urine samples. Congener analysis has often been utilized in cases where driving under the influence charges are refuted with drinking after the influence claims.[18,30] Congener analysis has been utilized in cases in Germany where drinking after driving challenges are used after vehicular accidents. This defense claims that the driver is not arrested immediately while behind the wheel but later, after an accident occurred,. In the interim, the driver consumes another alcoholic beverage. Current alcohol analysis is not capable of determining the differences between the two alcoholic beverages; but alcohol congener analysis can. The use of alcohol congener analysis has been able to distinguish between two alcoholic beverages effectively and successfully arguing against this "hip flask" defense.[18] Determining congener content and relating it to ethanol content as a ratio is the classic approach, this review investigates going beyond classical alcohol congener analysis by providing information about profiling and analyzing additional congeners.

Drinking alcohol after driving

In certain scenarios, when the perpetrator of a vehicle accident was not apprehended at the scene, a "hip-flask" defense if often used during prosecution. This defense challenges classic ethanol testing that has taken place by stating that there is no way to distinguish between ethanol consumed before or during the incident and drinking that has occurred after the accident has taken place. This defense is similar to a difficulty some victims face when after a DFC has occurred, the victim continues to consume alcoholic beverages to "calm" nerves.[18] This difficulty is where, despite the difficulties that may arise with congener analysis, this analysis will prove beneficial, shedding light on what class of beverage that was consumed before the DFC incident using classical congener analysis. There may be additional evidence regarding the identified congeners that the specific beverage consumed may be determined. Difficulties interpreting the results of congener analysis have previously been researched in the hopes of realizing half-life and other pharmacokinetic parameters of various congeners. These parameters have been predominantly investigated with congeners known as fermentation by-products, many of which

Citation: Mozayani A, Hohler MW, Drake J. A review of the use of alcohol congeners in the evaluation of alcohol facilitated sexual crimes. *MOJ Toxicol.* 2019;5(1):10–15. DOI: 10.15406/mojt.2019.05.00145

Copyright: ©2019 Mozayani et al.

are solvents with established pharmacokinetic parameters. These fermentation by-products are classical congeners that have been used previously, while ingredient biomarkers have more recently come to light and some still need pharmacokinetic parameters to be investigated. However, the use of both parts of the congener profile will allow for the investigation of beverages consumed.[18,31]

Fermented by-products

Fermented by-product congeners are formed during the fermentation process by the removal of the nitro-group of an amino acid and by replacing it with a hydroxyl group and creating the alcohol via the Ehrlich mechanism.[32] Fermented by-products can also include solvents that are present during the fermentation process. While initially it was determined that there were differences between different beverages (i.e. wine and brandy), when related to total ethanol content or absolute blood volume (ABV). It can also be determined for some congeners such as the isomers of butanol, that there is a statistically similar relationship. This creates a challenge for congener analysis because there is less differentiation between two beverages, especially when a standard drink is taken into account. Variation in higher alcohol congeners discriminates amongst Bourbons, Tennessee whiskey, Scotch and Irish whiskey, as well as single malt and blended Scotch whiskeys there are important differences in concentrations in blood.[26,33] Bonte published the initial report that congener concentrations in serum were different depending on the beverage consumed. For example, if drinks containing the same concentrations of three congeners, those with the highest concentration of ethanol gave the highest concentration of n-propanol, 2-butanol, and isobutanol in blood. Meanwhile, Bilzer et al. determined that one hour after drinking, the concentration of n-propanol in blood was five times higher and isobutanol was ten times higher than previously found.[14] It is important to be careful and to interpret these results correctly, because while it is easy to interpret that the sample was not out of the absorption phase, the differences in sampling (serum vs. whole blood) were the true difference between the two tests.[18] This leads to the importance of congener analysis being standardized, with validated methods. Bonte established many considerations for alcohol congener analysis, but the main inter-individual differences amongst individual elimination kinetics suggested that relating the results to the BAC allowed for more realistic evaluations of drinking after driving.[14] This also leads to the conclusion that relating congener concentrations to BAC in DFC investigations would be worthwhile. But, it also leads to the question as to whether the congener profiles alone would be useful. When drinking experiments are conducted, they offer the chance to evaluate first pass metabolism, as well as to estimate the small fractions of the congeners that arise in the bloodstream. This lead to two schools of thought arising when alcohol congener analysis in blood occurred: some researchers prefer information from drinking experiments, others used the modified Widmark formula previously discussed. This formula was used with known elimination rates and distribution volumes. With these variations to the established Widmark formula, it provides additional information that estimates congener concentration as it relates to ethanol content, but this information can also be compared to established congener profiles.[18,31] This combination of both schools of thought allows for metabolism changes to be discovered. This information assists in proactive DFC investigations by allowing for discrimination between two different alcoholic beverages. It also leads to the conclusion that by comparing to established profiles; it may be able to distinguish if another drug was added to the alcoholic beverage.

As the palettes of alcoholic beverages increase, the use of fermented by-products remains relevant, but the use of ingredient biomarkers allows for more concise information about alcoholic beverage consumption. The use of both of these profiles will allow more information to corroborate with witness testimony of what beverages were consumed. Ingredient biomarkers also increase the use of congener profiles when beverages such as vodka are consumed because they expand to flavoring agents that may be specific to the beverage in question.[31] Ingredient biomarkers may also increase the time available for testing (both sampling and storage) but may still have issues determining beverages within the same class. But, not unlike traditional ethanol testing, if the BAC is low, congener analysis may prove fruitless.[18]

Ingredient biomarkers

Ingredient biomarker congeners are congeners that are found in alcoholic beverages as a result of the ingredients and materials used during production. These congeners can include aldehydes, ketones, esters, histamines, additives, tannins, phenols, and organic acids. These congeners, which are more specific to the beverage in question, offer a chance to identify an unknown beverage if a victim of DFC encounters certain situations including a higher strength spirit being added to a lower strength beverage resulting in an increase in the ethanol intake of the victim (drink spiking), or when a victim claimed to have consumed a drink after the incident to calm their nerves, or when a concomitant drug administration occurs.[25] These biomarkers may also allow an advantage as alcoholic beverage palettes broaden with additional flavorings and increased aging times in some fermented beverages. Other acids including iso-α-acids (IAAs) are derived from the hop plant and are specific to beer. These acids are bioavailable and both *trans*- and *cis*- isomers can be monitored. The *trans*-isomers, being more water-soluble, allow for quantification, while the *cis*-isomers can be qualitatively monitored. While these IAAs are useful in their determination between low and high-hopped beer, it also provides some insight into their bioavailability as it relates to detection in blood. These acids were present as the *cis*-isomer at higher concentrations[33] These acids have also been determined to be stable up to 12 months in blood, and although concentrations remain low, with more sensitive methods, such as ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS-MS), it allows for separation and detection with little sample preparation.[34]

Additionally, xanthohumol (XN), isoxanthohumol (IX) and 8-prenylnaringenin (8PN) have been investigated as biomarkers for beer consumption. In a collection of randomized clinical studies, researchers learned that IX proves to be a valuable urinary biomarker specific to beer consumption because it is found in hops. IX recovery increased linearly with dose size in male volunteers, but a saturation effect was seen with female volunteers. Female volunteers excreted twice as much IX after the consumption of one beer, but no differences in IX excretion was found after the consumption of 1.5 or 2beers in a subgroup of female volunteers. The study also concluded that the excretion of some women is saturated at 152µg/mL in urine studies.[35] Additional research includes alcoholic drinks that are distilled, including spirits and certain wines. Research has also been conducted on absinthe, which contains thujones. Thujones are chemicals that are GABA-antagonists and present in absinthe from wormwood and other ingredients. These studies showed that it was possible to have high percent recovery with low error using SPME-

Citation: Mozayani A, Hohler MW, Drake J. A review of the use of alcohol congeners in the evaluation of alcohol facilitated sexual crimes. *MOJ Toxicol.* 2019;5(1):10–15. DOI: 10.15406/mojt.2019.05.00145

Copyright: ©2019 Mozayani et al.

HS-GC-FID to determine concentrations.[36] While it was important to understand these compounds within absinthe for authentication purposes, the linear range showed that sensitivity concerns are still present when using some of the ingredient biomarkers in biological matrices for headspace sampling. This hurdle may also be remedied with the use of a glass tight syringe on either a gas chromatography coupled with a mass spectrometer, or liquid chromatography coupled with mass spectrometry.[37–39] This allows for the sampling of headspace after proper sample preparation. Additional research is also needed to discuss stereo-selectivity of many of the congeners that would be useful in DFC investigations, but it is important to highlight because of how specific it then makes that congener presence. As was stated previously, if only *trans*- isomers were present, if *cis*-isomers emerged from a drinking study, it would bring doubt to any conclusions made from just IAA data.[35] By identifying congeners, it has been proven possible to determine if a stronger alcoholic beverage was added to a lower alcoholic beverage. This increased ethanol content may make the victim more susceptible to advances.

Another compound that could be useful as an ingredient biomarker is hordenine, which is a derivative of methyltyramine and an alkaloid in the β-phenethylamine class. Hordenine is present in barley, which is an important ingredient in many beers.[40] It progresses through the fermentation process but also has been present in serum samples; making it a valuable, discernible biomarker. Thereby, it would be useful to qualitatively determine a class of beverage and then continue to add specificity to that classification during quantification. Another question that arises is how to determine congeners from beverages that are distilled as well as fermented. Temperature influences the distribution of flavor active congeners between the solution and headspace, and longer aliphatic alcohols have different boiling points than ethanol, both instances will affect whether they are distilled during production. Fermented by-products and ingredient biomarkers that have similar boiling points to ethanol often remain in the distillate, but in some cases such as vodka, congener analysis remains difficult because so few distill with ethanol. In addition, studies have also shown that during maturation of model and malt whiskey, wood maturation changes key flavor components, and it was hypothesized that it would change it to the less desirable congeners in the liquid being stored. This adds additional specificity to the determination of congener profiles because some oaks, such as white oak, show fixed ratios of ingredient biomarkers, such as *cis*- and *trans*-oak lactones.[41] This gives validity to the use of ingredient biomarkers, especially stereospecific ones, which need additional pharmacokinetic parameters investigated to use the modified Widmark formula. However, the use of these ingredient biomarkers qualitatively would also give validity to the establishment of more complete congener profiles.

Postmortem considerations

One of the major criticisms of alcohol congener analysis is that it is often difficult to distinguish between congener alcohols and alcohols that have been created post-mortem. For example, Huckenbeck reported 1-butanol is often formed at the start of putrefaction, which is particularly created by bacterial decomposition. Previous studies done by this author, also determined that bacterial decomposition in human tissue did not lead to the generation of methanol. It remains important to collect blood samples correctly post-mortem, but also it would establish the need to use more than one category of congener analysis to determine if an alcoholic beverage contributed to the cause of death. Thus, by using qualification and quantitation, it will allow certain markers (ex. 1-butanol) to be used after a concentration that would be higher or lower than what could be determined as post-mortem neogenesis.[42] This expands upon the current congener analysis, relating congener content to ethanol content, but also using the congener profile as it relates to an established profile from a drinking study. If no drinking study is present, perhaps only a class of beverage may be determined. Ingredient biomarkers would also be important contributors, ensuring that specificity would be imparted. Also, it is important to remember that the volatile congeners are not only affected by phase I metabolism with ADH or other enzymes, they also are affected by phase II metabolism. This metabolism creates glucuronide counterparts as well as sulfate counterparts. These products have been used previously to determine if ethanol was consumed ante-mortem, and while these compounds have been investigated, no one has investigated whether the glucuronide or sulfates can be used to determine congener content as well.[43] This would allow the analyst to distinguish between which beverage was consumed when corroborated with witness accounts. If a DFC event proved fatal, fermented by-products and ingredient biomarkers would prove useful to investigators.

Alcohol congener profiles

With the use of fermentation by-products and ingredient biomarkers for the determination of what alcoholic beverage may have been consumed, there may be a direct link for DFC investigations to corroborate with witness accounts. While this may pose some issues logistically with alcohol congeners, it can be solved by using fermented by-products qualitatively and then by adding ingredient biomarkers quantitatively to complete the profile. Quantitation of congeners that are often in larger concentrations, will allow for determination of a complete profile for a beverage that has been ingested. While ingredient biomarker congeners have been proven to be the stronger link to alcoholic beverage consumption,[31,44] until further research is conducted, it will be difficult to differentiate alcoholic beverages within the same classes by using fermented by-products alone. However, using congener analysis the way that it has been accepted within parts of Europe, and then adding ingredient biomarkers allows congener analysis to prove useful as alcoholic beverage palettes expand and identification proves more difficult. This allows for the determination of beverages consumed based on congener concentration in a proportion with ethanol content, congener types, and congener concentrations would allow for determination of alcoholic class if not alcoholic beverage.

Conclusion

Combining various ways to perform alcohol congener analysis may allow for successful identification of specific markers that may identify a specific alcoholic beverage. Many factors including taking first pass metabolism into account, elimination rates, and half-lives have been investigated for specific congeners. As previously stated, these parameters allow for comparison between expected congener amounts in relation to ethanol content but also to complete congener profiles. These profiles become more complete by using ingredient biomarkers and more accurate as the use of the modified Widmark formula becomes more prominent.

Additional research needs to be conducted to determine a standardized, concise method that can be validated using strict Scientific Working Group in Toxicology (SWGTOX) guidelines allowing for more consistent analysis across laboratories.[45] While volatile congener analysis using HS-GC-FID allows for congeners to be added to routine alcohol analysis, GC-MS and LC-MS with a gas tight syringe would allow for headspace sampling with the increased

Citation: Mozayani A, Hohler MW, Drake J. A review of the use of alcohol congeners in the evaluation of alcohol facilitated sexual crimes. *MOJ Toxicol.* 2019;5(1):10–15. DOI: 10.15406/mojt.2019.05.00145

sensitivity of mass spectrometry methods. This increased sensitivity may be able to determine ingredient biomarkers that are found at considerably lower concentrations than fermented by-products. Presenting alcohol congener analysis during witness testimony shares some of the hardships of traditional alcohol analysis. This requires specialized training in alcohol toxicology to allow for successful communication about Henry and Ehrlich's laws, Richardson's law, and the details of the modified Widmark formula. Also, analysts will have to be able to explain the changes between the back extrapolation for ethanol content (BAC) at the time of stop, and the back extrapolation for each alcohol congener. It will also remain essential to maintain high quality assurance and quality control standards including the new standards in ISO/IEC 17025.[18–46] Additional research needs to be performed to ensure that congener profiling is successful. Many methods have used UHPLC-MS/MS to identify congeners, but Machata also formulated a way to see volatile alcohol congeners using HS-GC-FID. Volatile congener alcohols allow for congeners to be added to routine alcohol analysis, allowing for more efficient training, competency, and proficiency testing to be conducted. More complexity would be necessary for the quantitated profile, but would allow for HS-GC-FID to be an efficient screening test for volatile alcohol congeners.

Increased details for the back extrapolation would need to include explanation of the variability in epidemiological statistics of total body water volume, because many alcohol congener analyses use mean values, without incorporating any additional parameters. Not unlike alcohol analysis, the more pharmacokinetic parameters investigated for each congener allows for a more precise estimation of concentration at time of incident using the modified Widmark formula. Quality also remains a main topic that needs to be answered as alcohol congener analysis becomes commonplace in DFC investigations. The quality associated with DFC investigations have been at the forefront of news reports due to lagging backlogs for processing sexual assault kits nationwide.[47] While there have been various state reforms that have taken place,[48] congener analysis then becomes more useful for investigations that may have little evidence outside of witness accounts. Given that previous reports indicate that approximately 46% in some areas of the world have DFC using alcohol,[1] it is important to use alcohol congener analysis to corroborate witness accounts to ensure that no essential piece of supporting evidence is overlooked in a DFC investigation. In the United States, DFC is predominantly perpetrated with an alcoholic beverage of some kind. DFC investigation would benefit from this analysis by allowing the ability to determine definitively classes of beverages, but more complete profiles including ingredient biomarkers at smaller concentrations may be needed to discern beverages in a particular class. The combination of using fermented by-products to discern the class of an alcoholic beverage, and ingredient biomarkers to specify specific beverages in classes will allow for an additional advantage in opportunistic and proactive DFC to corroborate with witness accounts of what beverage was consumed.[49–53]

Acknowledgments

The authors would like to thank the support of the Texas Southern University Forensic Science Learning Lab and the Office of Research for SEED Grant funding.

Conflict of interest

The author(s) declares that there is no conflict of interest.

References

1. Anderson L, Flynn A, Pilgrim JL. A global epidemiological perspective on the toxicology of drug-facilitated sexual assault: A systematic review. *J Forensic Leg Med*. 2017;47:46–54.
2. Virginia Department of Forensic Science. *Toxicology Procedures Manual*. 2018. p. 8–218.
3. Collins DJ, Lapsley HM. Counting the Cost: Estimates of the Social Costs of Drug Abuse in Australia 1998-9. Canberra: Commonwealth Department of Health and Aging. 2002.
4. Widmark EMP. A micro-method for determination of ethyl alcohol in the blood. *German Biochem Z*. 1922;131:473–84.
5. Elbel H, Schleyer F. Blood Alcohol: the scientific basis of the assessment of blood alcohol evidence obtained from road traffic offenses. German. Second ed. Stuttgart: Georg-Thieme-Verlag; 1956.
6. Widmark EMP. The theoretical basis and practical application of medico-legal determination of alcohol. German. Berlin: Urban und Schwarzenberg; 1932. p. 1-140.
7. Machata G. The routine examination of blood alcohol concentration using a gas chromatograph. *Microchimica Acta*. 1962;50(4): 691–700.
8. Machata G. About the gas chromatographic determination of blood alcohol. Analysis of the vapor phase. *Microchimica Acta*. 1964:52(2):262–71.
9. Machata G. About the gas chromatographic determination of blood alcohol. *Blutalkohol*. 1967;4:252–60.
10. Machata G, Prokop L. About accompanying substances in the blood of alcoholic beverages. *Blutalkohol*. 1971;8:349–53.
11. Swift R, Davidson D. Alcohol Hangover Mechanisms and Mediators. *Alcohol Health Res World*. 1998:22(1):54-60.
12. McAnalley BH, Aguayo EH. The Chemistry of Alcoholic Beverages. In: Garriott JC, editor. Medicolegal Aspects of Alcohol 5th edn. Tuscan: Lawyers and Judges Publishing Co. 2008. p. 1–23.
13. Santillan valverde MC, Garcia-Garibay M. Biosynthesis of congeners during alcohol fermentation. *Rev Latinoam Microbiol*. 1998;40(1-2):109–19.
14. Bonte W, Decker J, Busse J. Congener Content of High Proof Alcoholic Beverages. *Blutalkohol*. 1978;15(5):323–38.
15. Bonte W. Congener Content of German and foreign beers. *Blutalkohol*. 1979;16(2):108–24.
16. Bonte W. Congener content of wine and similar beverages. *Blutalkohol*. 1978;15(6):392–404.
17. Bonte W. Alcoholic beverages impurities. Biogenesis, Occurrence, Pharmacology, Physiology, and Assessment. German. Lübeck: Schmidt-Römhild; 1987.
18. Iffland R, Jones AW. Evaluating alleged drinking after driving – the hip-flask defence. Part 2. Congener Analysis. *Med Sci Law*. 2003;43(1):39–68.
19. Posey D, Mozayani A. The estimation of blood alcohol concentration: Widmark revisited. *Forensic Sci Med Pathol*. 2007: 3(1):33–9.
20. Brick J. Standardization of alcohol concentrations in Research. *Alcohol Clin Exp Res*. 2006:30(8):1276–87.
21. Watson PE, Watson ID, Batt RD. Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr*. 1980:33(1):27–39.
22. Barbour AD. Simplified estimation of Widmark "r" values by the method of Forrest. *Sci Justice*. 2001:41(1):53–4.

23. Seidl S, Jensen U, Alt A. The calculation of blood ethanol concentrations in males and females. *Int J Legal Med*. 2000:114(1):71–7.

24. Watson PE, Watson ID, Batt RD. Prediction of blood alcohol concentrations in human subjects: Updating the Widmark Equation. *J Stud Alcohol*. 1981:42(7):547–555.

25. Staub C, Spörri AS. Ethanol and Drug-Facilitated Crime. In: Kintz, P, editor. Toxicological Aspects of Drug Facilitated Crimes. Amsterdam: Academic Press; 2014: p. 93–115.

26. Gonzalez Ariona D, Gonzalez Gallero V, Pablos F. Authentication and differentiation of Irish whiskeys by higher-alcohol congener analysis. *Anal Chim Acta*. 1999:381(2-3):251–64.

27. Simpkins WA. Congener profiles in the detection of illegal spirits. *J Sci Food Agr*. 1985:36(5):367–76.

28. Greizerstein HB. Congener contents of alcoholic beverages. *J Stud Alcohol*. 1981:42(11):1030–7.

29. Schreier P. Flavor composition of wines: A review. CRC Crit Rev Food Sci Nutri. 1979:55–111.

30. Jungmann L, Grosse Perdekamp M, Bohnert M, et al. Complex suicide by ethanol intoxication and inhalation of fire fumes in an old lady: Interdisciplinary elucidation including post-mortem analysis of congener alcohols. *Forensic Sci Int*. 2011; 209(1-3):e11–5.

31. Rodda LN, Beyer J, Gerostamoulos D, et al. Alcohol congener analysis and the source of alcohol: A review. *Forensic Sci Med Pathol*. 2013;9(2):194–207.

32. Hopfer H, Gilleland G, Ebeler SE, et al. Elemental profiles of Whisky allow differentiation by type and religion. *Beverages*. 2017;(3)8.

33. Rodda LN, Gerostamoulos D, Drummer OH. Pharmacokinetics of Iso-α-Acids in Volunteers Following the Consumption of Beer. *J Anal Toxicol*. 2014;38(6):354–9.

34. Rodda LN, Gerostamoulos D, Drummer OH. The rapid identification and quantitation of iso-α-acids and reduced iso-α-acids in blood using UHPLC-MS/MS; validation of a novel marker for beer consumption. *Anal Bioanal Chem*. 2013;405(30):9755–67.

35. Quifer Rada P, Martinez Huélamo M, Chiva Blanch G, et al. Urinary Isoxanthohumol is specific and accurate biomarker of beer consumption. *J Nutr Epid*. 2014:144(4):484–88.

36. Dawidowicz AJ, Dybowski MP. Fast determination of α- and β-thujone in alcoholic beverages using solid phase extraction and gas chromatography. *Food Control*. 2012;25(1):197–201.

37. https://www.agilent.com/cs/library/selectionguide/public/5990-3563EN.pdf

38. Jones AW. Biochemical and Physiological Research on the Disposition and Fate of Ethanol in the Body. In: Garriott JC, editor. *Medicolegal Aspects of Alcohol*. 5th edn. Tuscan: Lawyers and Judges Publishing Co. 2008:47–155.

39. Rodda LN. Alcohol Congener Analysis in a Forensic Context: Detection of Iso-α-acids to Confirm Beer Consumption. Ph.D. Thesis. Monash University, Melbourne Australia. 2014.

40. Steiner I, Brauers G, Temme O, et al. A sensitive method for the determination of hordenine in human serum by ESI+UPLC-MS/MS for forensic toxicological applications. *Anal Bioanal Chem*. 2016;408(9):2285–92.

41. Monica Lee KY, Paterson A, Piggott JR, et al. Origins of Flavour in Whiskies and a Revised Flavour Wheel: A review. *J Inst Brewing*. 2001(107)5:287–313.

42. Huckenbeck W. Neogenesis of ethanol and fusel oils in putrefying blood. In: Tsokos M ed. Forensic Pathology Reviews. Totowa Humana Press. 2007.

43. Hoiseth G, Berg-Hansen G, Mørland J. Evaluation of hip-flask defense by determination of ethyl glucuronide and ethyl sulfate concentrations in blood. *Forensic Sci Int*. 2015;257:398–402.

44. Kostik V, Memeti S, Bauer B. Gas-chromatographic analysis of some volatile congeners in different types of strong alcoholic spirits. *J Hygienic Engineering and Design*. 2014;4:98–102.

45. Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. J Anal Toxicol. 2013;37(7):452–474.

46. https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-3:v1:en

47. http://www.endthebacklog.org/texas-becomes-first-nation-implement-comprehensive-rape-kit-reform

48. https://www.forensicmag.com/news

49. http://what-when-how.com/forensic-sciences/congener-analysis/.

50. Iffland R, Jones AW. Evaluating alleged drinking after driving – the hip flask defense. Part 2. Congener Analysis. *Med Sci Law*. 2003;43(1):39-68.

51. https://www.agilent.com/cs/library/applications/5991-6638EN.pdf.

52. Anderson LJ, Flynn A, Pilgrim JL. A global epidemiological perspective on the toxicology of drug facilitated sexual assault: A systematic review. *J Forensic Leg Med*. 2017:47:46–54.