Just Analyzing Beverages for Cannabinoids Transcript

Introduction [00:00:05] Now, this is recording RTI Center for Forensic Science presents Just Science.

Voice over [00:00:22] Welcome to Just Science, a podcast for justice professionals and anyone interested in learning more about forensic science, innovative technology, current research and actionable strategies to improve the criminal justice system. In Episode 3 of the 2020 R&D season, Just Science interviews Dr. Carl Wolf from the Medical College of Virginia hospitals about 40 plus ways not to analyze beverages for cannabinoids. From professional student to a leading researcher in the analysis of cannabinoids, Dr. Wolfe has been involved in academia for decades. In that time, he has consulted and lectured on toxicology. Given expert testimony contributed to over 100 presentations and peer reviewed publications and worked on multiple and NIJ funded grants. Tune in as he talks about academia, analyzing beverages for cannabinoids and the value of failure in this episode of Just Science. This season is funded by the National Institute of Justice's Forensic Technology Center of Excellence. Here is your host, Dr. Megan Grabenauer.

Megan Grebanauer [00:01:34] Hello and welcome to Just Science. I'm your host Dr. Megan Grebanauer with the Forensic Technology Center of Excellence and Program of the National Institute of Justice. Today, our guest is Dr. Carl Wolf from the Medical College of Virginia Commonwealth University. He's been on the Just Science podcast before, an episode called Just Live or Die, where he discussed his other NIJ funded work about postmortem work and forensic toxicology. Carl, welcome to the podcast.

Carl Wolf [00:01:59] Good morning, Megan.

Megan Grebanauer [00:02:00] Good morning. So before you begin on your current NIJ funded work, do you have any updates on your live or die presentation?

Carl Wolf [00:02:07] Liver Doesn't Die? Yes. And other and other things we learned, well, that is actually after much prodding from students and fellows of mine, we submitted that last Friday for publication as a manuscript. So we'll see what comes out of that. We ended up with twelve different opiate assays that we evaluated commercially available ones and our own in-house developed one. And the data was kind of interesting as I presented at the meeting and I've presented it a couple other times is that none of them worked ideally for liver tissue. Surprise. On that one compared to urine or blood. But that if you understand the advantages and the disadvantages of each method, I think that most of them are useable in a postmortem liver doing liver analysis. They don't work the same as blood and urine, but they will do what you need to get out of it.

Megan Grebanauer [00:03:04] All right. Well, congratulations on getting the manuscript out the door.

Carl Wolf [00:03:06] Thank you.

Megan Grebanauer [00:03:07] It always feels good to put it in someone else's hands, then.

Carl Wolf [00:03:09] Yes, it does.

Megan Grebanauer [00:03:10] I see from looking over your bio that education is a pretty big part of your career. Is it still a big component of your daily work?

Carl Wolf [00:03:17] At one time, I was a professional student for many years of my life, and then it was one of those I want to say circumstantial things that happened in my life. Things came up and I didn't get my degrees when I got my bachelors and whatever, but my masters and then my Phd took a little longer than I had planned on. And then it was sort of like in that I had opportunities to take just a few extra classes like one hundred and forty four graduate credit hours of classes. So I've had the ability of taking the basic things that people take. But then I got to take a lot of odd and weird things which became and have become very useful in. And all the things that I do in my life, in my world. I talk to physicians and nurses and pharmacists and attorneys and judges and residents. And so being able to speak on their level is a great advantage. Then they're more willing to listen to you as not just you're the expert. Yeah. Yeah. Tell me what's going on. What if you can put it in their terms of what they understand? It makes it more personable I guess.

Megan Grebanauer [00:04:21] Did you take any particular classes that at the time seemed irrelevant, but it turned out to be pretty useful knowledge to have in your back pocket?

Carl Wolf [00:04:29] No, I. Maybe I'm too old or whatever. But you know, I had in high school we worked in chemistry labs that didn't have hoods. And, you know, we opened bottles on the counter. And I know there's safety issues and I understand. Trust me. I understand all that. I've yeah, blown up enough and burned up enough stuff in my life.

[00:04:48] I do. I do understand that. But some of that is the wow of that is what got me into that is the fact that, you know, you can you can watch things spontaneously combust or you can mix things together and make a new product in your life. This is kind of cool. And now a lot of that is done online. But you don't get to really see. You don't get to go, oh, what happens if I had a little bit more of this? What? You know, where does it go? Or if I don't put enough in? We only teach success. And in my life, failure has been far more often than what people call success. It's taken me a long time to get to where I am. And, you know, I wouldn't give up any of the any of the failures that have happened because that's how we learn.

Megan Grebanauer [00:05:34] I can tell you seem very comfortable with that. We'll talk about that in a minute. So we're here this week at the American Academy of Forensic Sciences annual meeting. You presented a presentation called Forty Plus Ways Not to Analyze Beverages for Cannabinoids. And that was part of the NIJ, forensic science, R&D and podium if listeners are interested in watching the archived recording of that presentation. It will be on the forensiccoe.org Web page or the landing page for this episode. Are there any other coworkers on this project that you'd like to acknowledge?

Carl Wolf [00:06:06] This would not have happened if it wasn't for our clinical laboratory science student, undergraduate student who we had work on this project. Heidi Breitman, because the university offers what they call UROP, which is undergraduate research opportunities program. UROP and they basically give students a stipend for the summer to work in their discipline. So this can be art. This could be history, social science, anything, not just. Science, science, they get to worked with somebody in the field working on a project and move beyond and get a better understanding of one, how to do research in their field, but also, you know, results that you can generate. And so I have a colleague, William Corazón, who's one of the faculty there, who I said, hey, do you have any students

for the summer? I got a project. And so he sorts through and goes, who's interested, who's not? And Heidi was like, I would like to do something for the summer. So I was like, cool. We have a cool project. Justin Poklus because he and I have been collaborating friends and collaborating for years on various projects. VCU and VCU Health and everyone else there that's sort of made this a possibility.

Megan Grebanauer [00:07:22] The title of your presentation to me is one of the more interesting ones I think I've ever seen in a program for a conference is being 40 plus ways not to do something. You're you're really laying it out there that this presentation is going to have a lot of negative results. And that's something you don't see too often. But it is debated in the scientific community is that we we tend to not disseminate or talk about things that didn't work. So why is it you felt it was important to bring that up here at the symposium?

Carl Wolf [00:07:51] You can thank my student, Heidi, for this one, because when she was working on it, she's like none of these work. And I'm like, oh, that's a perfect title. That's great.

Carl Wolf [00:08:00] And being a professional student for many years, I've seen lots of things that work and don't work. And I guess I'm not one to reinvent the wheel. I think there's far too many things that we do in this world that we end up reinventing the wheel because we don't disseminate information very well, whether it's positive or negative. You know, master students work on projects all the time and I'm involved with some of them and just all over the place where things don't work and that's OK.

Carl Wolf [00:08:27] But for your Phd Sorry, it has to work. And I understand that that's a great and wonderful thing. But when people publish things, they go, oh, we you know, we have a method for doing this. OK, but you didn't tell me what else you did to get to there. Or you maybe you said we did one or two things a little bit different, but no one tells you, you know, what the effects are of that. Because if I'm doing method validation, which is now a big part of everything we do, part of that is also, you know, can I tweak this one way or the other? What if I had a little more acid or a little more base or I add a little more solvent or, you know, what effect does that have? Do I change the P.H. a little bit? What effect does that have on my results? And that's really the more important thing because it talks about, you know, how robust your essay is. What can you do with it and what you can't do with it. So because this is a new field in the world of, you know, we've been doing drug testing and biologicals for years and you know, cannabinoids is a big thing. And in the food and beverage is becoming a very large part of the economy. But no one really knows what you're getting, you know. Now, people, I think, presume too much that the FDA slash some governing body is overseeing everything that's going on out there. And to my perception, that's not the case.

Megan Grebanauer [00:09:50] So let's talk a little more detail about the the goals of your project. Give us kind of in layman's terms, the big picture of what the purpose and the goals of this project you presented this week.

Carl Wolf [00:09:59] I'm sure this is a small part of a bigger project, as I said in the talk. The original premise was because this was before the impact, the Agriculture Improvement Act of twenty eighteen. My thought process on this was the fact that, you know what happens to soccer mom when she's bought CBD for her child who has epilepsy or something like that. And it works for the child and she's driving down the road and the police stop her for whatever reason that happens. And they go, what's in the car? And it's

like, oh, it's a CBD brownie. And the police at the time not understanding the difference between marijuana. Or she maybe she even said marijuana, Brownie, you send it to the lab and the lab goes, it's THC. Well, in Virginia, that's a schedule one. So that's a felony conviction right there, no matter how much is present in it. So I was like, I want to be on the defensive side on this one because I'm going to go. So how do you store these things? How do you test for these things, both by the officer and by the laboratory? And how do you know what's happened to this? And there's really there was nothing out there.

Carl Wolf [00:11:01] And I was like, this is going to become a big, major legal issue. And before it shows up on the 6 o'clock news in the morning news as this is the worst thing in the world and laboratories don't know what the heck they're doing. I wanted to, I guess, set the record straight from the beginning not to go back and have to fix this later.

Megan Grebanauer [00:11:20] So you set out to develop methods for measuring how much of the different THC and other cannabinoids are present and things besides urine and blood and the plant itself. But the cookies and the brownies and.

Carl Wolf [00:11:33] Correct.

Carl Wolf [00:11:34] Beverages came apart when the Agriculture Improvement Act occurred and suddenly it went from my local pulls food store of a little box on the counter that said CBD that was locked up to suddenly displays and aisles and it's everywhere. And I was like, this is going to be another problem. This is gonna be a big concern.

Carl Wolf [00:11:56] So why don't we go there?

Megan Grebanauer [00:11:58] Nice. And you managed to do it in one summer, the beverages portion?

Carl Wolf [00:12:01] Yes because we just did beverages.

Megan Grebanauer [00:12:03] Well, props to Heidi. She must have been very busy because that's a lot of work in a short amount of time.

Carl Wolf [00:12:09] She worked in a lab before she went back to school. So that was the nice thing about having that.

Megan Grebanauer [00:12:14] Where are you in the bigger project? When did it start and when do you expect it to end?

Carl Wolf [00:12:18] Funding officially started January 2018. And next month we will be doing the one year stability study of our homemade brownies. Gummies and dark chocolate, one year stability of our cannabinoids. The last analysis for the acid portion of that will be finished in the middle of March. So I will then be writing up my final summary to send to NIJ, then working on the manuscript to be hopefully out the door before the end of June when the funding officially runs out.

Megan Grebanauer [00:12:52] So in your presentation, which was specifically about the beverage analysis, you were you were looking at a way to compare different extraction methodologies and how to no one worked well and one didn't work well. And you use some terms. It's called matrix effect and process efficiency. Can you explain what those are for the listeners?

Carl Wolf [00:13:12] So a matrix effect is what is the matrix slash whatever your compound of interest is in, how much effect does that have on your analysis? Does it improve your detection of your stuff or doesn't interfere with it? Because in most things we do in the toxicology world, the compounds we're looking for are in miniscule decimal point percentages of what we're actually looking for, the needle in the haystack. So just that needle, how do you get rid of the whole haystack so you can only see the needle? And that's really matrix effect is how much does that haystack affect your analysis? And then the recovery is OK. How many needles do we really and we're really in that haystack. So do we get them all out or do we get most of that out? Theoretically, we should be able to get rid of all the matrix and recover all of our compound of interest. But we know in science that's not really a realistic possibility. So we work with what's acceptable. We accept ideally about plus or minus twenty five percent effect on over matrix. And our process efficiency is how well do we extract and clean up everything? And we'd like that to be 100 percent, but we know that it can be. We go with seventy five to one hundred twenty five percent to give ourselves some leeway to say, hey, this works or doesn't work. That's ideally in practicality because we're working with things that we have never or very few people have ever worked at. I use the philosophy if you can get in the end, get about 50 percent of what you're looking for. That's good. If you can't get half, try something else. There have been a few compounds we have tested in the past where 20 percent was the best. We were trying to do lipid filling compounds out of brain tissue. And that's not getting fat out of fat. It's not an easy thing to do.

Megan Grebanauer [00:15:03] So how do you actually measure these things in the lab?

Carl Wolf [00:15:05] We have a UPLC triple quad system that we use for all of our other opiate and cannabinoid testing. That's how we're doing them. It's probably a little overkill because most of these are in high concentrations. So we're ending up losing our samples in the end to some extent before we do the analysis. But you know, this is what we have. So you have what you've got. So as we do in all labs, we work with what we have. And, you know, if we get the opportunity that somebody wants to loan us or we have someone that we can collaborate with who has something else. The problem with cannabinoids are their schedule 1. So I can't just go, hey, can I send these over there for you to do the testing on? I'd love to. But besides federal. That's a university policy, right there.

Megan Grebanauer [00:15:53] Yeah. Researchers controlled substances. It does that does that is a hindrance sometimes. And who you can get to help you out and analyze and can receive the samples and work on it.

Carl Wolf [00:16:03] You didn't come to my world, but you know, nothing can leave here.

Megan Grebanauer [00:16:06] In order to accurately measure the extraction efficiency of all these different extraction methods. You had to prepare your own beverages that you know exactly what's in there, right. You can't say how well you took something out when you don't know what you started with. So can you talk a little bit about how you prepared the beverages?

Carl Wolf [00:16:22] The great thing about the Internet and students is that, you know, Heidi. And that's part of your the first thing we do is literature review what's out there, what's published, what have people done because. As they say, a lot of these people publish and go, we did this, OK, but what didn't you know, what did you do that you didn't work?

Carl Wolf [00:16:39] And maybe this is the best you got, but I'd really like to know how you got there, at least some summary of that. So serving sizes, all the important thing in the world of food. And so we have five and ten milligrams of THC, which is California on all those issues, Alaska, Oregon, Washington State, Nevada now. And we had a size of THC that we wanted. So the next question is, what are we putting that in? In the Brownie world, it was like, OK, Duncan Hines Brownie. And. They had a size that was like cool and Haribo and some other gummy makers had a size for that. And chocolate was Hershey's and Nestle. Like, what do you consider a size? Beverages was like, OK, they come in all different things and we went to the FDA and was like, OK, labeling of beverages. All right. And I was like, OK, so what are what are people selling and what are people buying?

Carl Wolf [00:17:32] People buy their twelve ounce can of Coke, Pepsi, whatever the heck it is. Mountain Dew, Dr. Pepper. You know, it's like and things come in 12 ounce. And if you get a larger size, they still usually correlate it back to a 12 ounce serving. We were like, OK, that is what we have a basis that everyone can understand. The perception is this is a twelve ounce as a serving. So that was our twelve on serving, which is three hundred fifty four milliliters. So now we have five milligrams in three hundred fifty four milliliters of beverage, whether it's soft drinks or pop, brewed things like because as the common is now it's in tea and coffee cannabinoids and people are making beer and wine. So we were like OK, there's our three matrices, sugar brewed and fermented.

Megan Grebanauer [00:18:20] How did you get the cannabinoids into the beverages? Because they're not typically soluble in things that are mostly water and beverages are primarily water.

Carl Wolf [00:18:30] Yes. And thankfully, they aren't primarily water does well for us. So we had to make an emulsion. And my student did various research of seeing what's out there, what are people doing? Because as I was like looking at this taking a pharmacy class, I understand the emotions and how they make compounding formulations. And I was like, huh? So we're making mayonnaise. That's what we're really making a suspension. Well, we don't want a suspension. We want to. We want them. So we wanted to go and stay as to the best. We're making salad dressing and we creamy Italian, not just Italian dressing. So why are people using out there to get the cannabinoids?

Carl Wolf [00:19:09] You know, you have to get that mysel partition. So you're gonna have the basis to understand where you're going. So fruit pectin seem to be what a lot of people were using. And people use fruit pectin all the time for canning. However, finding fruit pectin nowadays is not the easiest thing to do. There's only it's only got a few spots on a shelf because people buy pre packaged. And I was like, who? We can buy a box of schur gel or something like that. But that's powder. That's too hard. We have to weigh that. But there's a liquid form and I was like liquids.

Carl Wolf [00:19:41] Excellent. Because we're working with liquids. So we got that. We tried to we tried some variations on that and was like the one that was online. And it's on the presentation. The blog site was like it seemed to work the best that we saw, just mixing them up and getting it to stay as an emulsion for the longest time so we could do sampling out of it. Granted, as we left it set it separated, but for at least for 15, 20 minutes or so, half an hour, I think maybe it was the longest. We got a solution that was hopefully homogeneous. That's where that came from, was just trial and error and we just used their proportions.

Megan Grebanauer [00:20:18] Do you know, is that how the commercially available beverages are prepared or are they in emulsions?

Carl Wolf [00:20:23] Yes, they are. When I was in Seattle doing the liver talk, that was I went looking at the dispensaries because we were in Seattle. And what else do you do now besides. Yes. Trying the other things. And it was I was standing there looking at some of the beverages in the aisles. And the one gentleman came up to me who worked there and said, I've noticed you've been standing here awhile. It's like, yeah. And I explained to him, why was it because that's really cool? I said, you know, I'm just looking at your products and how these. And I was like, that's a suspension. And he's like, yeah, you have to shake him up to drink him. And I'm like, oh, okay. That makes sense.

Megan Grebanauer [00:20:58] Interesting. All right. So you had to buy the cannabinoids. Make the emulsions, add them to your beverages. So I know from my own experiences that buying cannabinoid standards can get pretty expensive pretty fast. Did you incorporate that into your experimental design or any strategies to kind of keep your costs down?

Carl Wolf [00:21:17] Yes. So that was that was the original the original thought process. We put money in the grant because standards we knew were going to be the most expensive thing for us to get. And that's a big part of our process, how we work that in there. One of the original thoughts was, oh, well, some of this stuff we could like buy online, like CBD, well, CBD was not available when you first started this online or any in any of the stores. They said hemp oil. But then there was a little disclaimer that this contains no phyto cannabinoids. So we're like, that's worthless.

Megan Grebanauer [00:21:48] Not going to help.

Carl Wolf [00:21:49] And, you know, you can get a number of things, but it's gotten. I will say in the last six months or year, it's gotten a whole lot easier to buy stuff. But we're on the back side, not on the front side. So we were purchasing them as certified standards. That's still an issue of buying any of these in labs. Trying to do this is how do you get referent enough referrence material, not just to make your calibrator, but to make your control materials too. Because most people go, oh, I want to make enough. That's going to last me six months or a year. Well, what has come with that is, is that we realize that we have serving sizes and we have sampling sizes. So we want to make our sampling, our proportion of our control not much bigger than our sampling size so we can freeze them, slash whatever store them. And that's part of the stability process going on. And what's the best way to store these things with beverages? We ran into the issue of cannabinoids are not very stable in beverages unless somebody knows something or maybe it's the way we prepare them, whatever. But our high sugar content products, cannabinoids within and in less than a week were not really detectable out of the nine. We looked at. And the alcohol in the fermented lasted at least up to about 10 days. We didn't do any testing beyond that because the summer was over and we still have some in the refrigerator and maybe one at some point in time, Heidi, you'll be able to come back as part of her spring semester. She's got a couple of days and we'll run a batch of those and see how they look six months later. But we noticed that right away because we would make a batch to do our effects, to look at the effects. And she would be like, this isn't working. And I'm like, did you make it fresh? Because now I made a big batch. And, you know, we're running for a couple of days and it's like it's not working.

Megan Grebanauer [00:23:39] So you had to make fresh batches each day for extractions?

Carl Wolf [00:23:43] For doing the matrix effect and process efficiency, yes.

Carl Wolf [00:23:47] When we did our method validations, we did the calibrator is fresh every day and then our control materials will run as stability issues. We ran those. We made them up and stuck them in the refrigerator because nobody really freezes these things. And we left them out on the counter and we didn't see much difference between that and refrigerated. So it was like, okay, just keep them in the refrigerator.

Megan Grebanauer [00:24:08] All right. So how did it go when you tried 40 things that didn't work? Did you ever find anything that did work?

Carl Wolf [00:24:14] Yes. Well, we found two things that worked. One, there's a paper out there published on doing it by methanol and just doing a methanol dilution and running it. And that's why we do this, is to look not to debunk people's things, but to go. OK, how does this how is ours better? Because you're never going to paper published unless you're better than someone else.

Carl Wolf [00:24:36] That's just something unspoken rule. So, you know, we did methanol and what we saw was our process efficiency on this because we're just eluding them was about 20 percent overall. Three matrices we looked at, which I go.

Megan Grebanauer [00:24:52] So that means you're only able to get out 20 percent of what you know you put in.

Carl Wolf [00:24:57] Or the effect the appearance of. Yes. It appears that you only got about 20 percent of your compound out. So whether that's that you had so much suppression of it from the signal that maybe you got 100 percent, but you've got so much suppression that you didn't get to see it or you truly only got 20 percent out. That was an issue. But we were like, okay, if that's the best we can do, then that's what we have. The problem we ran into as we were doing fruit drinks as methanol dilution doesn't get rid of all the pulp and all the other crap in there and it doesn't centrifuge out either. So now you've got to go to another step and you've got to filter it or you've got to run it on your instrument and hope that you don't plug it up. And I've already plugged my instrument enough times over the years to go, that's not going on my instrument.

Carl Wolf [00:25:45] And now we're doing a two step process. So now we're diluting and filtering. So what do we just do that together? Keep it simple. In for laboratories doing this testing if it's a 10 step process. I don't see most forensics, government, whatever, you know, private labs going. We're not gonna spend all day to do an analysis. We want something that's simple, simple and quick to do sample prep, throw it on the instrument, and if it takes five minutes, two minutes or an hour to run the samples. So what, we're going home. We'll pull them off the next day and we'll crunch the data and get it. So that's mine is how fast can we work on it. But get a method that works, really. What we found that was work because we had them from a project from eight or 10 years ago was the United Chemical Technologies fastcolumn. It's proprietary, so they won't tell you what's in it, but they have what we call a green and a black column. That's the labeling on the tube. That's all. Green light green. The green one we know is for acid and neutral extracts, and the black one is for a basic neutral extracts. So most people use the black column because those drugs are bases and we compared those in the initial work on this and

doing gummies and they worked really well. They had similar comparisons when we did Sugar content things in high sugar content. We ran them on our chemistry analyzers and it's like, oh, the sugar is gone. Awesome. This is great. So we had some previous experience with it in my one student at the time presented at at the academy meeting. And it worked well. And the really was the only difference we saw between the two columns was the Green column did not take out our rude red food coloring, whereas the black one did. And I'm like, OK, so if there's color left, then something is not taken out of it. When we looked at it for doing beverages, we didn't see any difference with color. But what we saw a big difference was was sugar content. So sugar is the hardest of the beverages to analyze. Of the three, it has the most matrix effect and it has the most ion suppression both ways.

Carl Wolf [00:27:58] It enhances your signal in some cases and it depresses your signal in other cases and compared to the other two. And as I'm looking at this, as we did with the other edibles, is when samples show up in your laboratory, you don't some you don't know right off the bat what it is.

Megan Grebanauer [00:28:13] Right. It doesn't come with a nice little tag. That says exactly what you're analyzing.

Carl Wolf [00:28:16] So when we were doing the edibles, parson, my student was like, well, let's do this and this and this. And I said, no, they all have to work together. I said, because what do you do with the chocolate chip cookie? I said, better yet, what do you do with a chocolate chip cookie with gummy worms in it? You can't pick out the parts and then figure out which one has the cannabinoids in it. It's as a the law is written as total content. So you got to grind the whole thing up and analyze a portion of that beverages people make. You know, people put sugar in their tea and then their coffee. You don't get to choose what's in there and you can even have Irish coffee or whatever you want to call it, you know. So you end up with this mixture of all three common matrices that you have to run into. So your method had better work for all three of them or it's not really useful.

Megan Grebanauer [00:29:04] How many individual methods do you think it's realistic for an analytical lab that's going to go into business or a publicly funded lab? Can you see me analyzing these edible products? How many different methods do you think they'd be willing to implement for this analysis? Do you think they would? If it's more than one, they're they're not going to do? What did you think about that? One for solids, one for liquids.

Carl Wolf [00:29:26] I think that's what you're gonna have to do, because when you do in the biological world, you still have to do some kind of sample preparation on your liver tissue before you can add it to your urine and blood test. So, you know, that's still two tests. Must there's something else coming up? Yes. If you want to do food and we'll just use food as a general thing if you're. Because we don't have to deal with gases too much in food. The original elements, solids and liquids, you're gonna need to have two different methods because they don't act the same.

Megan Grebanauer [00:29:59] So in the results you presented, it was it was fairly common and even the extraction methods that didn't work out so well. Yeah, your bread and fermented beverages seemed to track pretty close like they had similar performance. And then the high sugar ones were either way, way better or worse than that. What is it about this high sugar things? Do you think that might be interfering or causing that discrepency?

Carl Wolf [00:30:20] Perceent sugar content.

Carl Wolf [00:30:23] I think because sugar is such a big molecule and sugar is such a large portion of this, we didn't do anything like distilled spirits for our for our alcohol. We were doing what's classified as beer. So things between about three and a half to maybe seven or eight percent alcohol. We did a couple of wines, too, so we were in the 12 to 15 percent brewed. Ah, you know, it's whatever tannins and slash, slash, whatever is coming out of the coffee bean or whatever your plant material is. So the content is not that high. It's probably I don't remember the amounts, but it's, you know, 90 some percent water with flavoring, slash colorings in it, alcohol. You kind of have the same kind of thing. You're in the 90 percent, 80 something within your wines that it's water with sugar content foods. The sugar is a high proportion of read, the label is a high proportion or sugar is a big molecule to compared to small molecule. So it has a large effect on what's going on. We can't. We ran a couple sugar free things to. We saw sort of in the middle. So it's probably, you know, some of those are fairly large molecules when you think about it compared to something like an ethanol molecule.

Megan Grebanauer [00:31:43] So I think they had artificial sweeteners instead of sugars?

Carl Wolf [00:31:44] Artificial sweeteners too. Yeah, we didn't see as bad an effect, but it wasn't as good or it wasn't as consistent. I want to say with, say, with the brewed in the fermented somewhere in the middle. So that was like, OK, then that was better going. Look, let's just deal with true sugary drinks. And some of those were generic and some of those were brand names. We looked at about 12 or 15 different things on the market and made our own concoctions in them and saw some that some that worked better than others. And that's my thing is I think it's just the fact that you're dealing with just the pure size of trying to clean up that much of whatever it is in there and get rid of it.

Megan Grebanauer [00:32:26] So what's next for your research? She mentioned you've got some final one year stability time points coming up. But what's the next phase for you?

Carl Wolf [00:32:34] My funding ends in June this summer. Hopefully, I have to. I might have three students coming to work on emerging opioids and fentanyl analogs. So the CDC commissioned surreally and came in chemical to make opioid and emerging fentanyl analogs.

Carl Wolf [00:32:58] Maybe this is my perception, but I thought we were supposed to get a method along with the standards. Maybe I read something wrong a long time ago and that's like I say, my perception. But I have the standards, but I have no real method and no one's really published anything other than, you know, a case where they said, Oh, we did this or that by whatever method they used. So we have no way to screen for two hundred and twenty some compounds and really see if they're what's going on out there. So what I have a couple of students this summer come work on is that they were going to develop an LCMS method for screening because the quantitation of these in a person is it's there and you're dead. Wow. That's not a you know, that's not a big leap of faith anywhere, especially if it's the only thing or one of a few things present in there. Wow. That's pretty simple. But, you know, how can we screen for these? So initially, we need to literally infuse these and figure out how to chromatograph quickly, separate them because a lot of them are isomers of each other. Isobars and you're going like mass spec. A simple molecular ion is not going to do this. Like I said, I have a couple of students working on this and this

is our summer project is to come up with a I'm going to say hopefully a method, but I'll be really surprised.

Megan Grebanauer [00:34:21] Wow. Very ambitious.

Carl Wolf [00:34:24] You know? Shoot high and then drop on back, but maybe two or three different things or an initial, hey, this is how we can screen for all these. We may not be able to distinguish X, Y and Z compounds, but if there's something there that eludes at that time period and has this similar molecular fragmentation, that it's one of two or three things. And then you can go. OK. Now let's take that because it's a screening technique and push it and figure out which one it is. If you care. So that's that's the process. And where it's becoming important is this is funny, you know, coincidence. Things are med talks. Folks in the E.R. department guys said, hey, we need somebody to do testing for new fentanyl analogs out there on the market or not in the market that are happening because we're a level one trauma center. And being from Richmond, you're on ninety five. So everything from Miami to Boston stops in Richmond at some point along the way. You know, what they're seeing in their patients is that typically a patient on an opioid gets a shot in Narcan and you wake up and hit Fenton or heroin has been very big enrichment for years. And then in September of 2016, fentanyl started getting cut into our heroin. And what they've been seeing with this is not just fent, no fentanyl analogs is. So patients get a shot of Narcan or whatever in the field. They kind of wake up, they bring him in, they're out again. They give him another shot. They wake up. And if it's just heroin, that's usually enough for the patient. Then observe them for several hours and then they come back and they recover and they're cognizant and they release them. But what they're seeing now with patients is as they come in or they get a shot of Narcan or two in the field, they give them a couple of shots in ED and sometimes they even have to hook them up to a Narcan drip and then they get admitted to the hospital. And now you're dealing with patients that are they're not responding as quickly as they would with heroin.

Megan Grebanauer [00:36:18] It takes a lot more resources?

Carl Wolf [00:36:19] And it takes a lot more resources and time. And so they're like, can we figure out a way to test for this? And maybe we can come up with a scenario of boom. If the patient comes in and you get him a couple of shots of Narcan and they don't wake up or they kind of do, sort of a retrospective back look and go, OK. These patients need admitted to a ward slash unit and they need to be maybe on Narcan or maybe they find out that something else works better afterwards as to treatment protocols for these patients. And I said, oh, we got these two hundred and some standards. This would be cool. We could work together on this because they're like, we don't want to quantification. We just want to know, is it there or not? And I said, perfect. This is what I want to work on also. So we're waiting and working on IRB approval. I need to have a method. So that's that's the new research project.

Megan Grebanauer [00:37:11] So how convenient. When your funding runs out, you'll have time to work on that.

Carl Wolf [00:37:13] Yes, exactly.

Megan Grebanauer [00:37:15] Well, there's always a silver lining.

Carl Wolf [00:37:16] There is. And sometimes people step back. Take a breath. Not a bad thing and go, OK. So where do I need to go now? Because obviously straight ahead is not going to work.

Megan Grebanauer [00:37:27] So that's all we have time for today. I'd like to thank our guests, Dr. Carl Wolf, for sitting down with Just Science to discuss his NIJ funded grants. Thank you, Dr. Wolf.

Carl Wolf [00:37:36] You're welcome. It's my pleasure.

Megan Grebanauer [00:37:38] I'd also like to thank you, the listener, for tuning in today. If you enjoyed today's conversation, be sure to like and follow just science on your podcast platform of choice. For more information on today's topic and resources in the forensic field, visit forensiccoe.org. I'm Megan Grabenauer. And this has been another episode of Just Science.

Voice over [00:37:58] In the next episode, Just Science Interviews, Dr. Tatiana Trei hosts from West Virginia University about the rapid detection of organic and inorganic firearm discharge. Using laser induced breakdown, spectroscopy, opinions or points of views expressed in this podcast represent a consensus of the authors and do not necessarily represent the official position or policies of its funding.