04

QUALITY CONTROL IN THE CANNABIS AND PHARMACEUTICAL INDUSTRY

INTRODUCTION

For the pharmaceutical industry, the majority of drug candidates explored in early development will never make it to clinical testing, let alone the market – and the point at which a candidate is abandoned may follow years of work and investment. Tackling this inefficiency, through better drug candidate identification, optimization and selection, could have a major positive impact on drug discovery, to the benefit of pharmaceutical companies and patients alike.

At Pittcon every year, pharmaceutical and technology industries gather to hear about some of the major developments assisting effective early drug development.

Additionally, there will be a unique opportunity to hear from the Enabling Technologies Consortium (ETC), a collaboration involving leading pharmaceutical companies, who are joining forces to tackle the industry's needs in a mutually beneficial way.

The rise of automation

In recent years, the rise of robotics and automation within the pharmaceutical industry has had a major impact on early drug development. High-throughput screening complements advances in genomics and chemical synthesis to allow scientists to screen molecules against potential therapeutic targets per day. The technology accelerates drug discovery, reduces costs and has led to drug discovery in otherwise neglected disease areas.

The endurance of liquid chromatography

Liquid chromatography-mass spectrometry first came about in the 1970s, but developments in the technique continue to fuel pharmaceutical research today. With trends towards increasingly complex samples, biomedical scientists need ever better ways to prepare and separate them. For example, polymer conjugation has become an important approach in drug development. It aims to alter the pharmacodynamic or pharmacokinetic characteristics of a drug by conjoining it to a polymer, such as polyethylene glycol.

Collaborating on success

The development of enabling technologies, such as instruments, reagents and computer models, poses a challenge for pharmaceutical companies, particularly at a time when they are under pressure to reduce operating costs and improve productivity. Increasingly, companies are joining together in so-called precompetitive collaborations to tackle mutual needs and find solutions that can work across the industry, rather than just for one company's needs. This approach has successfully been applied in semiconductor, transportation and other industries; what could it hold for pharma?

In a bid to find out, a group of pharmaceutical companies formed the Emerging Technologies Consortium (ETC). Through the formation of workgroups, they aim to tackle specific priorities in pharmaceutical chemistry, manufacturing and controls. They hope that the approach creates a less legally complicated means for pharmaceutical companies to collaborate and, when they do, allows them to share costs as well make sure they are addressing the most pressing needs of the industry.

4.1 ANALYSIS AND AUTOMATION TECHNOLOGIES FOR PHARMACEUTICAL RESEARCH

High-throughput screening (HTS) has become a cornerstone of pharmaceutical research in the post-Human Genome Project era. The approach allows the rapid analysis of 1000s of chemical compounds at a time, something which has been instrumental in identifying small-molecule drug candidates, for which genomic research throws up dozens of targets. HTS first gained traction as a drug-discovery tool in the late 1990s and several drugs have since come to the market that owe their existence to the approach.

HTS has a number of advantages for the pharmaceutical industry. The method employs robotics, detectors and software to screen and assay a large number of chemical compounds against a target, sometimes ranging to 100,000 per day. This has created huge potential for accelerating drug discovery, particularly given that chemical synthesis can generate huge libraries of novel compounds.

HTS can reduce the costs of drug development and has facilitated drug discovery efforts in previously neglected disease areas. It also allows for the early determination of compound toxicity.

The emergence of HTS as a critical tool for drug discovery has been underpinned by developments in robotics and automation. At Pittcon 2018, delegates were able to meet the companies who are developing the latest technologies for use in HTS. David Damon from Pfizer Inc will discuss the development of workflows for the HTS of Lewis acids. These acids are routinely used in the development of chemical processes to prepare active pharmaceutical ingredients.

Also at Pittcon 2018 were Life Technologies, part of ThermoFisher Scientific, who have developed an online web portal called SelectScreen, which allows their customers to view real-time screening data. Typically, when scientists outsource compound screening and profiling, it takes around 5-15 days for a report to be delivered to the client. With the SelectScreen workflow, the team at Life Technologies have shown that clients receive 75% of their data on average within the first two days.

Pittcon also featured numerous exhibitors providing a range of automated instruments that can be applied in HTS including Metrohm International. Their automated sample preparation system, the 815 Robotic Soliprep, can be used for a range of applications, including in ion chromatography, HPLC, ICP, voltammetry and titrimetric applications to ensure uniform sample preparation at every step. Their scientists have demonstrated that the instrument can be used for the homogenisation of pharmaceutical tablets, as is required for accurately quantifying the ingredients and verifying the active pharmaceutical ingredient.

Beckman Coulter provide a range of integrated automation solutions to assist optimizing lab throughput. Their Biomek automated liquid handlers allow lab scientists to reduce their hands-on time during drug discovery research and can be integrated into complete workflows, including from third party devices, such as bar-code readers, microplate centrifuges and temperature-controlled storage.

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4.1a DEVELOPING A HIGH THROUGHPUT MASS SPECTROMETRY PLATFORM FOR DRUG DISCOVERY

Over the past 20 years, electrospray ionization mass spectrometry has been applied widely across areas including proteomics, metabolomics and imaging. In drug discovery, the qualitative and quantitative approach offers a sensitive, reliable and robust tool for studying molecules of interest at low concentrations, as well as macromolecules that are difficult to study with other techniques.

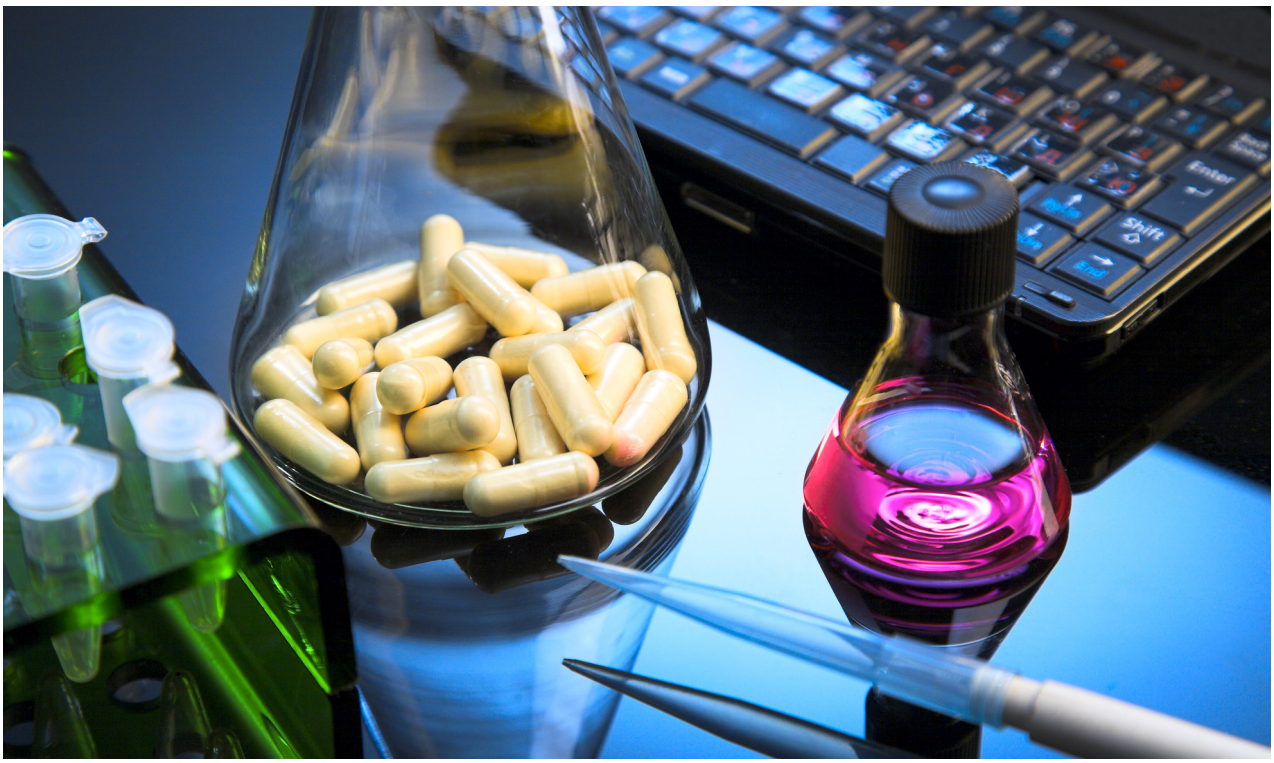
Electrospray ionization involves spraying a sample using an electrical field, leading to the formation of charged droplets. Following desolvation, the ionized analytes can then be detected with a standard mass spectrometer.

Electrospray ionization involves spraying a sample using an electrical field, leading to the formation of charged droplets. Following desolvation, the ionized analytes can then be detected with a standard mass spectrometer. At Pittcon 2018, Jonathan Wingfield from AstraZeneca discussed a collaboration between the company with Labcyte and Waters to develop a high throughput mass spectrometry platform.

It uses acoustic ion generation, similar in principle to electrospray ionization, which the team refer to as Echo-MS. It involves a modified acoustic liquid handler which ejects a mist of droplets directly from a 384-well assay plate. These are ionized as they pass through a transfer tube which delivers them into the mass detector.

The team have already demonstrated that it could screen 300,000 samples during a single assay, using manual plate loading and they have now adapted it using an automation to feed plates into the system, allowing a screen of around two million samples.

In this interview conducted at Pittcon 2018, Dr. Wingfield discusses the high throughput data so far collected using this prototype system and shares recent work demonstrating how it could be applied in drug discovery.



Your presentation at Pittcon 2018 focussed on Mass Spectroscopy (MS) based high-throughput screening for drug discovery. What are the current challenges associated with screen samples?

Acoustic mass spectrometry offers us the ability to screen very, very quickly in a very clean way. This is a huge benefit for the high-throughput screening community. As a result, we are able to have a direct measurement of substrate to product conversion, and that's great because we don't have to buy extra reagents. We also don't have to buy labels, which obviously add cost. Higher costs mean we can only screen a reduced number of samples. So, keeping the cost down is important for us too.

In a recent paper co-authored by yourself and Mattias Rohman, you describe the use of RapidFire™ coupled to a triple-quadrupole MS for high-throughput screening. How does this compare to traditional liquid chromatography for electrospray ionization (ESI)?

In the high-throughput screening space, the RapidFire platforms that are now marketed by Agilent are considered to be state of the art for high-throughput screening. It is because they are using a simple solid phase extraction methodology, rather than an LC, that means they can process a sample every eight to ten seconds. However, even at that speed, it's really not fast enough. Of course, taking away the LC component, to a certain extent also demystifies some of the technology around mass spectrometry and makes it a little bit more user friendly as well.

Can you please tell us about your collaboration with Labcyte and Waters to build a prototype high throughput mass spectrometry platform?

"The aim of the collaboration that AstraZeneca has with LabCyte and Waters is really to try and push forwards the boundaries of high-throughput mass spectrometry."

Dr. Jonathan Wingfield

Electrospray mass spectrometry is broadly applicable across may areas of drug discovery, and although there are some other high-throughput techniques such as MALDI, many labs will probably have access to electrospray mass spectrometry platforms.

So being able to convert those into a high-throughput technology was really the main aim for this. The use of acoustics is exciting because it gives us an opportunity to use all of the advantages of acoustics, low sample handling and very high-throughput potential, in a way that's not been used in terms of coupling it to mass spectrometry in the past.

What modification have you made to the acoustic dispenser and what have been the benefits?

Fundamentally, we've taken the LabCyte 555 and essentially taken the transducer from inside and brought it outside the instrument, then coupled this to a plate transfer system so that the plate can now move around over the top of this transducer.

The frequency of the transducer has been tuned and the power offset is subtly different from a standard two and a half nanoliter droplet. This enables us to generate a droplet that's much smaller and therefore more straight forward to ionize. It also gives us better signal in the mass spectrometer itself.

What is the Echo-MS system and what sampling speeds have you been able to obtain?

By coupling the acoustic front end to a standard time of flight or triple quad mass spec, we can achieve throughputs of around three samples per second. When you compare it to the state of the art electrospray system, the RapidFire, which processes samples once every eight to 10 seconds, it gives us a considerable speed advantage. At that sort of throughput, we're able to process more than 100,000 samples per day and half a million samples per week.

What level of sample screening do you hope to achieve at these levels?

Well so far, within AstraZeneca, we've been able to use this technology even though it is still in its prototype stage to support our high-throughput screening campaigns. Last year we ran a full collection screen, 2.2 million samples, and today we've probably processed around about 5 million samples through the platform. This is something we've never been able to do in the past with a single MS system.

Why is this important to drug discovery in general?

"One of the things that it really enables us to do is to build the assays very quickly. We can get a very fast proof of concept using the mass spec to see whether our enzymes can convert substrates into products."

Dr. Jonathan Wingfield

Once we've established that proof of concept, moving from there to having a proper assay fit for high-throughput screening is a relatively quick process.

Of course, as I said, costs really come down as well without having to invest in additional label it brings the prices down to a much more affordable level. It's also a very simple process. We simply add the reagents to our plates, and we have the reactions occur, and then the same plate is used to fire samples into the mass detector itself. For example, our last high-throughput screening campaign was probably about 90,000 pounds cheaper than if we had screened using traditional label-based technologies.

What are the next steps in the development of this prototype platform?

We're still working with prototype systems. I don't even believe they're beta products yet, they're still in the alpha stage. As with any type of research project like this, I think it's probable that we will get significant improvements in sensitivity, which will really add value as we move much more towards a commercial instrument.

I think we're still a year to 18 months away from having any kind of commercial offering available, but hopefully we'd like to see it broadly applied in the biochemical screening space. We're now starting to broaden our view to look at other potential application avenues as well. We'll be sharing some data here around cell based assays, which would be a first application with the acoustic mass spec in that area as well. We're very excited about that and hopefully we'll see more applications in the future.

Why is Pittcon important in helping you share your research?

Pittcon is really important to us because it gives us a really great opportunity to talk to a really broad audience. Pittcon's renowned for being at the forefront of analytical sciences, and acoustic mass spectrometry is just another analytical tool. Being able to come to a conference such as this one, and be able to talk and access such a wide spectrum of scientific excellence, I think is really important for any project.

Waters, whose mass spectrometry devices were used in the prototype were also at Pittcon 2018.

Their MS instruments enable maximal performance in pharmaceutical analysis from pre-clinical testing to clinical trials. Scientists from the company have recently demonstrated how a high-sensitivity method coupling UPLC to tandem MS could be used to study the pharmacokinetics of the asthma drug fluticasone proponate in the blood serum of rats, even though it circulates at concentrations as low as 10 pg/mL.

Liquid Chromatography/Mass Spectrometry in The Tharmaceuatical Industry

Liquid chromatography- tandem mass spectrometry (LC-MS/MS) has become a mainstay at all stages of pharmaceutical development. The expansion of mass spectrometry into the industry was accelerated by the advent of electrospray ionization, a nobel-prize winning achievement that allows for the ionization and analysis of large bio-molecules.

Exhibiting at Pittcon 2018, ThermoFisher Scientific offered a range of LC-MS systems, software and accessories.

Scientists from the company have recently shown the potential of paper spray technology for fluid analysis. The direct-ionization method could provide a simpler and more rapid method for studying blood and urine samples, in clinical research, forensic toxicology and in anti-doping in sport or horse-racing, without the need for extensive sample pre-treatment and chromatography.

The team from ThermoFisher tested the approach using horse urine samples spiked with methamphetamine and other stimulant drugs. Using a triple-quadrupole mass spectrometer, they were able to perform quantitative analysis of seven different analytes.

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4.1b MARKET HIGHLIGHT: THE OUTSOURCING OF PHARMACEUTICALS

Patent expirations, growth of disease prevalence, and the greater availability of advanced diagnostic procedures have increased the discovery and development of new biopharmaceutical compounds at an exponential rate. Pharmaceutical outsourcing has become a massive industry and represents a key driver behind many of the drugs brought to market today.

Why Are Pharmaceutical Companies Outsourcing?

When it comes to pharmaceutical research and development however, success is never guaranteed. The average cost of drug development can reach into the billions of dollars, and it takes an average of 10 years to bring a new drug to market. In addition, many drugs fail to be clinical effective and subsequently never see the light of day.

Due to these risks, pharmaceutical companies are not always eager to utilize their onsite resources for drug R&D. Certain cost-saving measures implemented in the pharmaceutical space have included outsourcing these activities.

Helping organizations reduce both their operational and manufacturing costs, thereby lowering financial risk.

Current outlook

An estimated $19.2 billion dollars were spent in 2016 for outsourcing the discovery of pharmaceutical drugs, which was primarily driven by a greater demand for contract research organizations (CROs) as well as advancements in scientific discovery and technology. Additionally, there is an expected growth of 46.7% from now until 2021 in the development of new biologic entities, translating into an overall expenditure of $72.7 billion.

Currently, the most common pharmaceutical products being outsourced for development include:

- Vaccines (51%)
- Blood factors (46%)
- Hormones (44%)
- Antibody drug conjugates (42%)

Specifically, drug discovery outsourcing is expected to rise to a $43.7 billion industry by the year 2026. The outsourcing sector is saturated with an enormous amount of capital, which continues to fuel pharmaceutical outsourcing.

Since many pharmaceutical companies are small, have little capacity to perform research onsite, and lack appropriate equipment to perform R&D, outsourcing is essential.

As evident by its exponential growth, R&D outsourcing activities have become challenging to manage by pharmaceutical technical and R&D managers. Private contract research organizations (CROs) and other academic organizations represent the primary outsourcing locations for pharmaceutical companies, and many of these companies provide everything from drug discovery and structural analysis to phase 4 clinical trials.

Drug development

Pharmaceutical outsourcing is typically allocated to CROs, specifically in the testing phase. Prior to ultimate market approval, clinical trials are required to be carried out in controlled environments. This is where the CRO comes into play. In addition, pharmaceutical companies may wish to outsource their sales and marketing to external vendors as a means of improving efficiency and optimizing expenditures.

ThermoFisher Scientific, a leading biotechnology product development company who exhibited their range of spectroscopy products at Pittcon 2018, is an example of a company that provides outsourcing drug development services to pharmaceutical clients across the globe.

While not a CRO, pharmaceutical companies can still utilize ThermoFisher's services for identifying drug compounds via cell- and biochemical-based profiling, assaying compounds or formulations, and gaining assistance in cell engineering projects. Pittcon 2018 exhibitor Bruker, also offers pharma-development services - including drug discovery and drug development programs, N and C terminal sequencing, structure determination via single crystal X-ray diffraction, cell culture media monitoring, and X-raying services.

In addition, Waters, a biotechnology instrument manufacturer, also assists companies in developing drug formulations, peptide and protein bioanalysis, and other manufacturing services for the biotech, pharma, and health sciences industries.

Pharmaceutical testing

One of the biggest challenges associated with pharmaceutical development is that of biopharmaceutical analysis. Onsite analyses are possible for large biopharmaceutical companies; however, the costs, time, and equipment associated with these tests can exert a substantial burden on the company's resources.

ThermoFisher is specialized in the field of biopharmaceutical analyses as they offer a full suite of technological products that assist in rapid, high-quality analyses of minute pharmaceutical compounds. Many of their technologies aid protein characterization, assist in identifying three-dimensional structures, and facilitate protein aggregation.

Additionally, ThermoFisher provides pre-clinical and clinical drug testing, including drug metabolite characterization to facilitate new compound discovery and drug development. The organization relies on high-resolution mass spectrometry technologies for detecting, profiling, and quantifying metabolites.

Additionally, the company includes a quality assurance and quality control protocol which consists of multiple technologies, including mass spectrometry, to ensure consistency and to detect impurities in pharmaceutical samples.

Electron paramagnetic resonance (EPR) spectroscopy is an essential aspect of pharmaceutical testing, helping researchers gain insight into the drug's degradation.

By doing this, pharmaceutical companies understand the shelf life of a product, which ensures patient safety.

Outsourcing pharmaceutical development organizations, like Bruker, offer this service in addition to their other development programs. Also, nuclear magnetic resonance imaging can work synergistically with EPR spectroscopy to assist in discovering insight into the structure of impurities, potency determination, and structure of biological drugs and biosimilars.

In addition to understanding a drug's shelf life and potency, a drug stability test chamber is often used by pharmaceutical outsourcing organizations, like Hanon Instruments, to determine a structure's strength under varying environmental conditions. Another organization, DynaLabs, provides stability indicating tests to determine the stability of pharmaceutical compounds.

CONCLUSION

Future innovation in pharmaceutical development relies on a more focused approach that moves away from competition and into areas where unmet needs exist. Additionally, pharmaceutical companies should realize the increasing rarity of blockbuster drugs and should focus on a strategy that pursues targeted therapies to diversify and increase portfolio value. In addition to outsourcing R&D activities, these actions will ensure greater cost-savings and higher returns on investment.

Outsourcing with experienced leaders in pharmaceutical development, such as ThermoFisher Scientific, Waters, Bruker, DynaLabs, and Hanon Instruments, will help ensure these goals are met now and in the future.

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4.2 CANNABIS QUALITY AND CONTAMINATION TESTING

In this interview, Dr Bob Clifford from Shimadzu, describes the importance of analytical testing in the Cannabis industry and the techniques involved:

Why is analytical testing important in the cannabis industry?

Just as government agencies such as the FDA, USDA, and EPA require testing of food, medicine, and lotions we put in or on our body, cannabis should have those same requirements, as it is consumed. This is especially true for medical cannabis patients who may have an immunocompromised system.

How can cannabis become contaminated or unsuitable for its intended use?

There are many contaminates cannabis consumers don't want in their product; these include pesticides, residual solvents, heavy metals, mycotoxins and microbial pathogens.

Contamination of cannabis can be by intentional or unintentional means. Examples of unintentional contamination could be from toxic heavy metals like arsenic, cadmium, lead, and mercury from the soil or water used to grow the plants.

Another example of unintentional contamination is with pesticides, which can be harmful if they enter the body. "Pesticides" is the collective term used to describe pesticides, herbicides, insecticides, and fungicides. There are currently more than 1000 pesticides in the world today. So, while pesticides poison pests and increase crop yield, they can also poison humans as well.

For example, the fungicide myclobutanil is commonly used on commodities such as almonds, asparagus, and grapes. An overspray of this on a commodity in a field next to a cannabis farm, could spread to the cannabis plants itself. If the fungicide is exposed to a flame (i.e. when the cannabis is smoked), it can produce hydrogen cyanide gas – a gas poisonous to humans.

Alternatively, it is possible that the pesticide may be sprayed on the cannabis intentionally, to increase crop yield. In a recent survey of cannabis flowers analysed from a US state, Shimadzu found 15% of the dried flowers had residue from the myclobutanil.

The number increased to 38% in concentrates since not only are the cannabinoids concentrated, but so are the pesticides. Further, in the same study, there was some type of pesticide residue on 52% and 82% of dried flower and concentrates.

What are the different analyses involved with cannabis testing? What components of cannabis are involved in these?

There are +500 compounds which should be tested during the analysis of cannabis. The main components include cannabinoids, terpenes, amino acids, proteins, glycoproteins, enzymes, sugars, hydrocarbons, and phenols.

The cannabinoids and terpenes are of special interest because of the synergistic effect they have, called the "Entourage Effect", which refers to the combined effect of the different compounds present in that particular type of cannabis. The profiles of the cannabinoids are important to patients as each one has different medicinal properties, so they will need to know which is best suited to their medical condition.

The terpenes are reported to have their own health benefits and thus the profile of those are of special interest as well. There are more than 120 terpenes reported to be in the cannabis plant.

Terpenes can be found not only in cannabis plants, but also other botanical sources. For instance, in a pine forest one can smell pinene. Relative to cannabis, pinene acts as a bronchodilator opening the lungs for better cannabinoid absorption.

Another example of a terpene is Linalool, found in lavender. It has a floral smell and acts as a sedative, and is reported to have anti-cancer properties. Limonene, in citrus fruits, has been reported to have anti-bacterial, anti-cancer, anti-depression, and anti-fungal properties.

Solvents such as butane and propane are used to extract the cannabinoids from the plant. The leftover toxic chemicals after the extraction are referred to as residual solvents. The cleanest way to extract the cannabinoids is with supercritical fluid extraction (SFE), which generally uses carbon dioxide and has the status as generally recognized as safe (GRAS) by FDA for use in foods. It is non-toxic and not flammable, unlike solvents.

Heavy metals contamination can come from soil, water or air. The "Big Four" toxic heavy metals are arsenic, cadmium, lead, and mercury. States such as Maryland have added barium, chromium, selenium, and silver to the "Big Four" list for cannabis testing while New York has a different set to the existing list such as antimony, chromium, copper, nickel and zinc.

Mycotoxin traces its roots to the word "myco", which is Greek for fungus, and "toxin" for poison. Mycotoxins are secondary metabolites from the fungus and can cause disease and death to humans.

Aflatoxins are a type of mycotoxins produced by certain molds, specifically Aspergillus species of fungi, and can cause liver damage and cancer. Foods have allowable very low levels of mycotoxins, which also should be true for cannabis consumed by patients.

The final type of cannabis test includes that for microbial pathogens, such as E. coli, salmonella, and listeria. Fifteen pathogens in food cause 95% of the hospitalization with salmonella being the most prevalent with 19,000 hospitalizations costing $3.7 billion annually, according to the USDA.

"By using analytical techniques to test cannabis for microbial pathogens, we are able to avoid a situation like this in the cannabis industry."

Dr. Bob Clifford

Please outline the techniques involved in cannabis testing. Are the same analytical techniques used for each type of test?

"High-performance liquid chromatography (HPLC) with a UV (or PDA) detector is used to test the cannabinoids with Shimadzu's Cannabis Analyzer for Potency."

Dr. Bob Clifford

If the analyst is thinking about increasing the number of cannabinoids tested, then a triple quadrupole liquid chromatography mass spectrometer (LC-MS/MS) or high-resolution mass spectrometer is required.

In contrast, terpenes are typically measured by a headspace GC-FID or headspace GCMS. The headspace is an accessory for sampling the cannabis by heating the sample in a vial and collecting the volatiles in the gas phase for introduction to the gas chromatograph (GC).

The GC-FID is a gas chromatograph with a flame ionization detector, while GCMS is a gas chromatography mass spectrometer. The GC-FID measures the retention time the terpene enters/exits the detector after being separated from other terpenes and volatiles by the chromatography column.

So, if two compounds have the same retention time, they would be indistinguishable by the headspace GC-FID. The preferred method is GCMS as a library search can be used to give a fingerprint of the specific terpene.

Pesticides are typically analysed by a triple quadrupole liquid chromatography mass spectrometer (LC-MS/MS). Some compounds are difficult to ionize by LC-MS/MS. As the pesticide list varies between states/countries, it is subject to change yet still and therefore the addition of a triple quadrupole gas chromatography mass spectrometer (GC-MS/MS) may be required for complete pesticide analysis.

Heavy metals are usually analysed by cannabis labs using an inductively couple plasma mass spectrometer (ICP-MS). The ICP is an "electrical flame" at 10,000 degrees, as hot as the sun, and ionizes the metals to be detected by the mass spectrometer. These instruments can analyse more than 70 elements simultaneously within a minute so if additional heavy metals to the "Big Four" list are required for testing, then there are no problems.

A limited number of mycotoxins in foods can be analysed by HPLC with a combination of UV and fluorescence detectors. However, because of the complexity of the cannabis plant, with more than 500 compounds, an LC-MS/MS is required.

This can be the same LC-MS/MS used for pesticide analysis with sample preparation and a different chromatography column for separating the mycotoxins/aflatoxins.

Microbial pathogens can be found by a variety of methods, each with its advantages and disadvantages. Enzyme-linked immunosorbent assay (ELISA) technology has a low upfront cost of $10-$20 per sample for rapid foodborne pathogen detection based on antigen/antibody-binding but may be limited to a single microorganism and can have false positives and negatives.

Quantitative polymerase chain reaction (qPCR), based on DNA may be in the $20,000-$40,000 range and can expand the number of microorganisms tested.

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) is the top-of-the-line technique routinely used in clinical settings, but just starting to make it to the food industry.

The upfront cost of the instrument is expensive at more than $300,000, but the advantages are numerous with routine analysis of greater than 1,300 microorganisms in less than two minutes for pennies a sample, without false positives and negatives based on protein analysis.

"Pittcon 2018 will demonstrate all the latest instruments suitable for the above techniques, and will have information on the technologies in the cannabis testing industry."

Dr. Bob Clifford

Is it compulsory for all cannabis to go through these tests?

As cannabis is illegal on a federal level in the US, there are no federal regulations for testing. It is up to each state to determine which test will be implemented and enforced, and these vary between states.

Take pesticide testing, for example. California tests for 66 pesticides, Oregon tests for 59, Nevada tests for 24, but some states may not have a pesticide program in place at all yet. Keeping track of each state's ever-changing cannabis testing program can be very time consuming especially as links change and become inactive.

It has been announced that jCanna will be presenting a Cannabis Symposia at Pittcon 2018. What are the key points that you will be sharing here?

As a youngster my father told me tools are 90% of the job. The same is applied to cannabis testing, and this will be my story to tell at Pittcon 2018.

I will discuss the quality control and labelling of cannabis, and how this varies from state to state and country to country - whether it is protecting brand name, providing customers with more information, or educational research, a number of analytical tools are utilized.

As I described before, the tools or analytical instruments depend on the testing to be completed, identified, and quantified. Therefore, the seminar will describe which analytical instruments are required for testing each of these parameters.

"My seminar titled "A Survey of Analytical Instruments Used in the Cannabis Testing Industry", is part of the half-day cannabis session taking place on the morning of Monday, February 26, 2018."

Dr. Bob Clifford

Please outline the recent legal and ethical developments in medical cannabis research.

Cannabis focus is not just quality control (QC) testing anymore, but also R&D and education. For example, Northern Michigan University offers a degree in Medicinal Plant Chemistry with an emphasis on cannabis, herbal extracts, and natural product industries.

Shimadzu is the only manufacturer of analytical instruments for testing cannabis products that also has a medical division. One of the products includes functional Near Infrared Spectroscopy (fNIRS) where patients with Post-Traumatic Stress Disorder (PTSD), concussions, or chronic traumatic encephalopathy (CTE) brain images could be monitored to understand the effects of cannabinoids on the patient.

How do you think analytical testing of cannabis will develop to keep up with the "legalization era"?

I believe that it is time for the federal government to step in and set up federal guidelines for cannabis testing to prevent citizens from harm due to intentional or unintentional producers, processors, or dispensaries. After all, more than half the states in the USA now have a medical marijuana program.

4.2a REASSESSING POTENCY ANALYSIS

Investigation of Interferences and Method Specificity Using Liquid Chromatography-Ultraviolet Detection and Tandem Mass Spectrometry

Potency analysis methods typically consist of a simple sample preparation, which includes extraction and dilution, before LC-UV analysis. This usually involves a basic set of steps, e.g. weighing, grinding, extraction, diluting and filtration, to produce the flower extract for potency analysis. The biggest problem faced when carrying out potency analysis is inconsistencies between analysis of the same samples but carried out at different laboratories.

Demands such as a potency number to two decimal places, which are typical of companies with no scientific background, don't understand that with every method and test that is carried

out, there will be variation, a percent error in that value. Therefore, a potency number of 22% could potentially be 21%, statistically, and so giving decimal values is meaningless. And so, it is also important to have an education component when working with our clients.

The methods themselves must also be considered i.e. are they functioning the way that we think that they are? Especially as this is a fairly new industry, it is worth considering whether the methods can be improved.

As new methods get developed, and different laboratories begin to use them, variations in same sample results can help us learn and improve these techniques. It can be seen if they are not functioning in the way that they were intended to for example, which is all part of analytical chemistry.

Changes in the market that effect potency analysis directly. Initially samples brought in were typically flower plant material or concentrates, then edibles were also frequently brought in and now more recently analysis of formulated products are being requested. Formulated products are samples that have come from for example cannabinoids independently formulating them, as well as taking synthetic terpenes and adding them back in synthetically. The methods that have been developed for analysing potency of concentrates and plant material may not be appropriate for these new types of samples, formulated products, that are more commonly being brought for analysis. So, these were the motivators behind this project of reassessing and improving potency analysis.

When I joined the cannabis testing lab, a year ago, as a scientific director and began this project, I was looking at the method reassessment from an analytical chemist with a chromatography background. Our potency

testing method was 17 minutes, which is too long, especially from a commercial and financial point of view.

One of the challenges for this particular assay is due to the way that our chemical standard has to be received, and so they are limited in concentration.

Therefore, we are frequently having carry out multiple mixes at a single calibration point, as well as having a wide range and so having to carry out many calibration points. This means that just the calibration curve itself, if you have a long run, can take up to half or a whole day. This made speed a priority in the reassessment.

A second motivator for reassessing potency analysis is around the lack of evidence from systematic studies supporting its specificity and the findings from Amanda Rigdon, which showed that under certain chromatographic conditions there are interferences. There will be other compounds contributing to the signal measured and that will give an elevated value. In chromatography, you are able to adjust the method to solve this type of problem.

However, even under optimized conditions, interferences were occurring. There was the possibility that this could be as a result of terpenes colluding with the cannabinoids, but because they are typically only present in very low levels in the plant they should not contribute significantly to the signal, when looking at such a high level of cannabinoid.

However, now that clients are bringing in samples that have had synthetic terpenes added into the products, they do become an issue in regards to interfering with the signal. The theory was that terpenes would not respond well by UV. Therefore, in this project individual terpenes of full spectrum calibration were evaluated in order to find out if and where they respond, and ultimately try to figure out where the problems are going to occur. Then, we compared this to a more selective method that used mass spectrometry (LV-MS).

Chromatography

The method we have now is 9 minutes and we can currently do 11 cannabinoids and report out 10, because there's one that we monitor because we know it can potentially interfere. We are hoping to create a method that allows for 14 cannabinoids.

We also wanted to create a method which was isocratic, although this might be controversial, we wanted the baseline to not adjust from the effects of UV on the baseline.

We also wanted to create a method which address the issue of terpenes and selectivity, and we went from a fixed wavelength UV to a diode array detector, which give you some different capabilities. Starting with the chromatography, we evaluated five stationary phases. We've looked at various mobile phases, methanol, acetonitrile, different additives, different pHs, as well as different flow rates and column temperature. And finally, we also evaluated different dimensions, which involved getting a set of columns in standard dimensions to do column screening, and then once making a determination on the final stationary phase, a couple of different dimensions were analyzed.

Chromatography...so far

Column : InfinityLab Poroshell 120 EC-C18 (Agilent), 3.0 x 100mm, 2.7micron
Mobile Phase : 28% water (0.1% formic acid), 72% acetonitrile (0.1% formic acid), isocratic
Column : 1mL/min
Temp : 30°C

Compound	RT (min)	Compound	RT (min)
CBDVA	1.1	CBN	3.3
CBDC	1.3	d9 THC	4.2
CBDA	1.7	d8 THC	4.4
CBGA	1.8	CBL	5.1
CBG	1.9	CBC	5.6
CBD	2.0	THCA	5.9
THCV	2.2	CBCA	7.0

14 cannabinoids

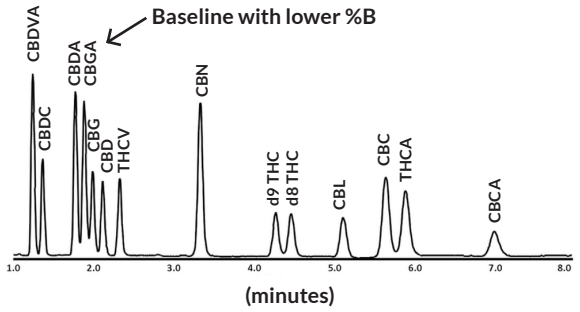


Figure 1: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Sample preparation

Our standard certified method for processing the samples was used, which is very straightforward (figure 2). Depending on if it's a concentrate or flower we use 0.1 and 0.3g respectively. The samples are immediately dissolved in 20 mL of methanol, then filter and do another 1:10 dilution, and that will go on liquid chromatography with ultraviolet detection (LC-UV), or diode array. MS/MS is more sensitive, and so we carried out an additional 1:100 dilution, and then in some cases from that an additional 1:100. Some of the samples were still off scale.

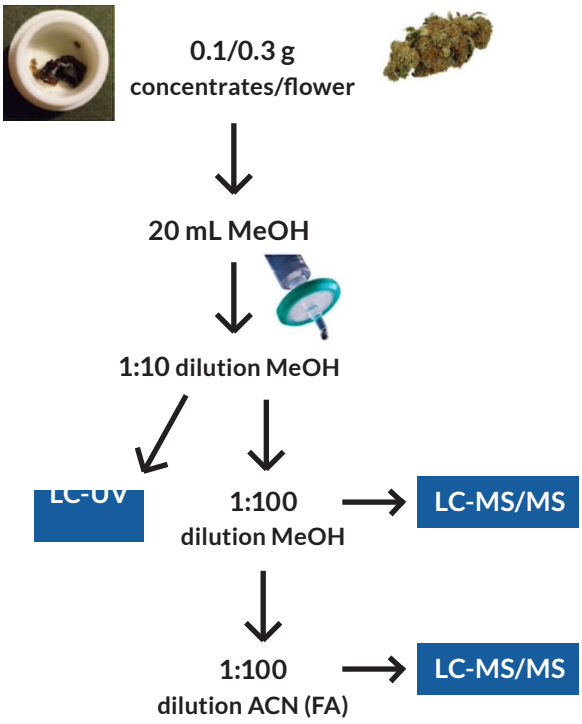


Figure 2: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

LC-MS/MS

Shimadzu Nexera XR UHPLC
SCIEX Triple Quad TM 6500+

Compound	RT (min)	Compound	RT (min)
CBDVA	1.2	CBN	3.7
CBDV	1.4	d9 THC	4.8
CBDA	1.8	d8 THC	5.0
CBGA	2.0	CBL	5.8
CBG	2.1	CBC	6.4
CBD	2.2	THCA	6.7
THCV	2.5	CBCA	8.0

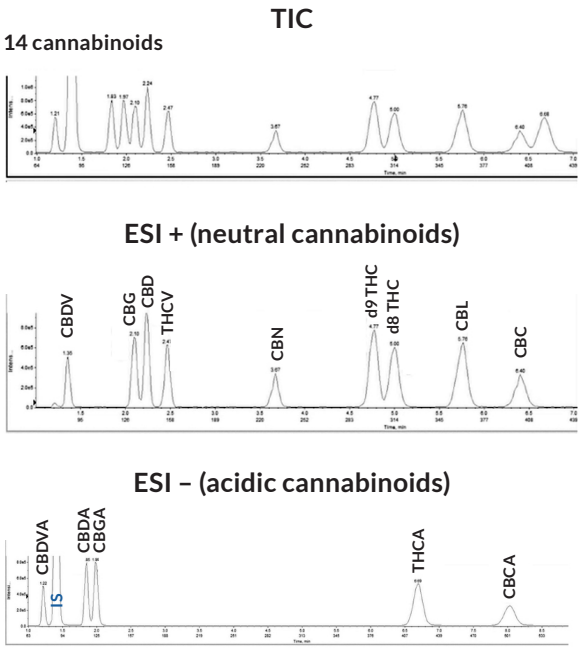


Figure 3: *Example of sample using LC-MS/MS. Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

38 terpenes with cannabinoids MRM method

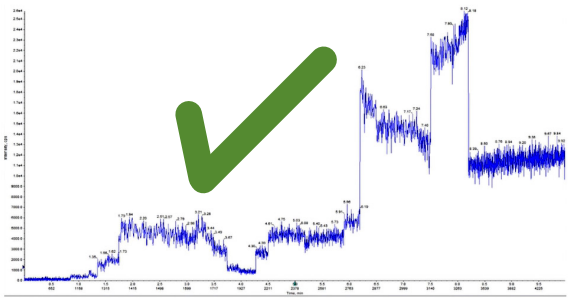


Figure 4: *High level terpene. Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Diode array detector

Using the diode array detector was a different for us and it has some additional capabilities. You can look at full spectra of multiple wavelengths. If there is a colluding compound, you carry out compound suppression, which is similar to a background subtraction for a specific compound.

Below in figure 5 is our method for 10 ppm cannabinoids is shown in green and 10 ppm terpenes is shown in purple, which is approximately 38 terpenes. This is at wavelength 228 nm which is where our current method is that we run on a day to day basis, you will see indicated by the yellow circles that there are some terpenes. They are causing an issue as people are adding synthetic terpenes back into sample these signals have become much more disruptive.

LC-UV (single wavelength)

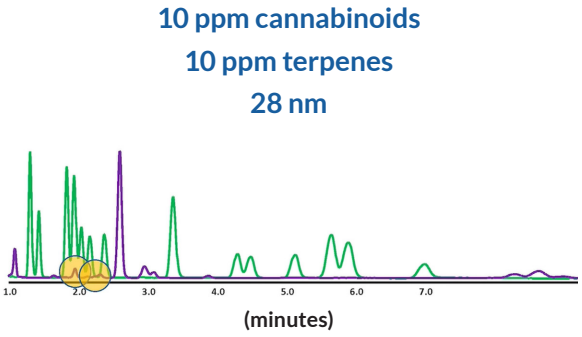


Figure 5: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Looking at figure 6, this is the full wavelength spectrum of alpha-pinene, we were focusing on where there is large signal, and where there is not a signal and understand these characteristics. If overlay all the terpene signals, which can be seen in figure 7, terpenes tend to have signal in the low nanometer range.

LC-UV (DAD)

Alpha – pinene

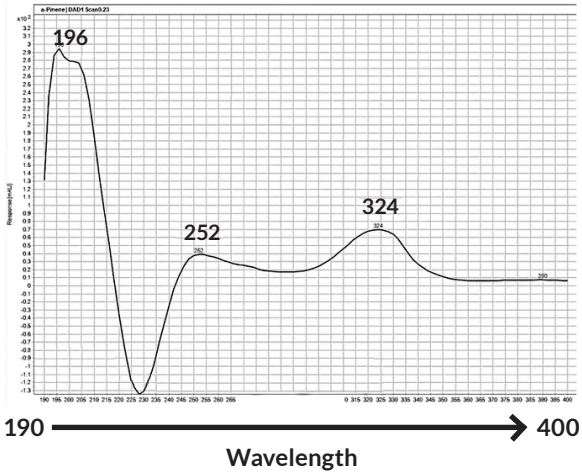


Figure 6: *A full spectrum, so this is simply the wavelength of alpha-pinene. Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Terpenes

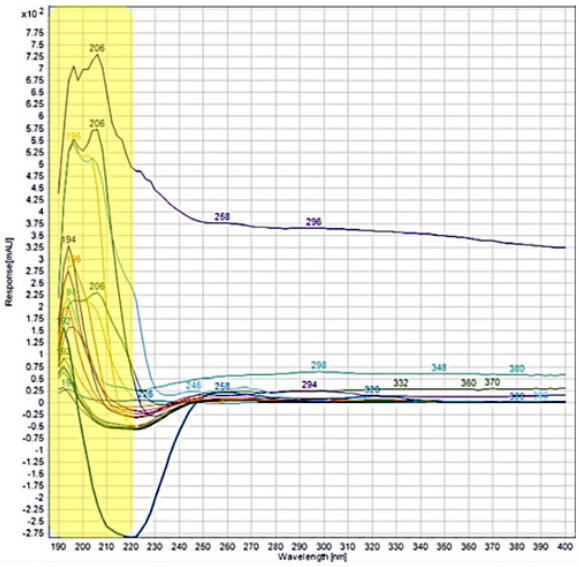


Figure 7: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

LC-UV (DAD)

Delta-9 THC

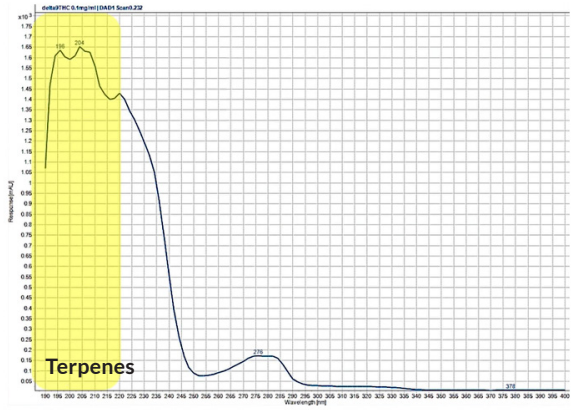


Figure 8: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Neutral cannabinoids

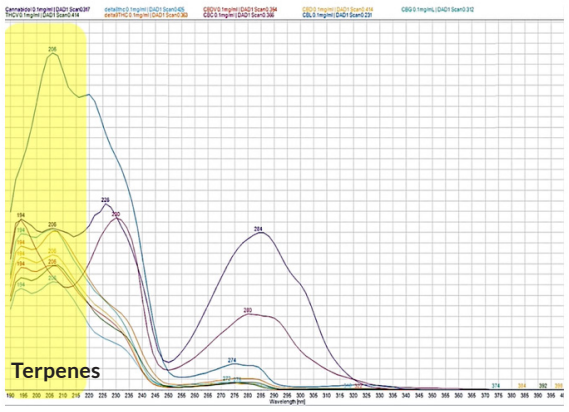


Figure 9: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

CBDA

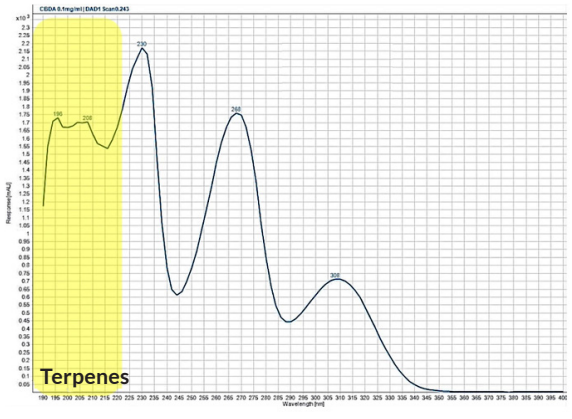


Figure 10: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Delta-9 THC (figure 8), you can see that the terpene range is in the area where it has its high signal, as expected. But there are also other opportunities (signals) that can be detected for this compound. To see this better, if we overlay all of the neutral cannabinoids (figure 9), you can see that there's two general regions, as well as a lot of fine structure being present. Therefore, the terpenes, to some degree, eliminate this region from use. We also looked at CBDA, an acidic cannabinoid, (figure 10) and it has a very different and more interesting profile. Terpenes again overlay, but there are also a lot of other areas in this spectrum wavelengths that can be used to identify it.

When overlaying the acidic cannabinoids with the terpenes, we found that there was still lots of additional peaks to identify it with. This means that there are a lot of other compounds in this extract, and when there is this many, it becomes difficult to remember all the overlays and what to do etc. and so, I came up with a system.

Using CBDA as an example here, I begin by plotting a dot, darker larger to lighter in terms of the maximum wavelength (figure 11), and I do that for all of the cannabinoids. The axis here is just the names of the analytes.

Then I take the first maximum wavelength, and plot it for each one of the cannabinoids, and figure 12 is what it's going to look like. Then the second, third and fourth (if present) maximums are also plotted. This gives a map of where the potential signal I want to look at could be and enables me to identify and select the low sensitivity and my highest intensity and narrow down the possible candidate wavelengths (shown in figure 12 C in green shaded boxes.

CBDA

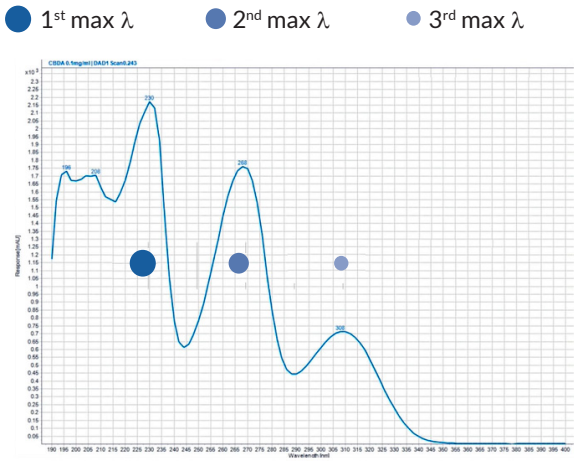
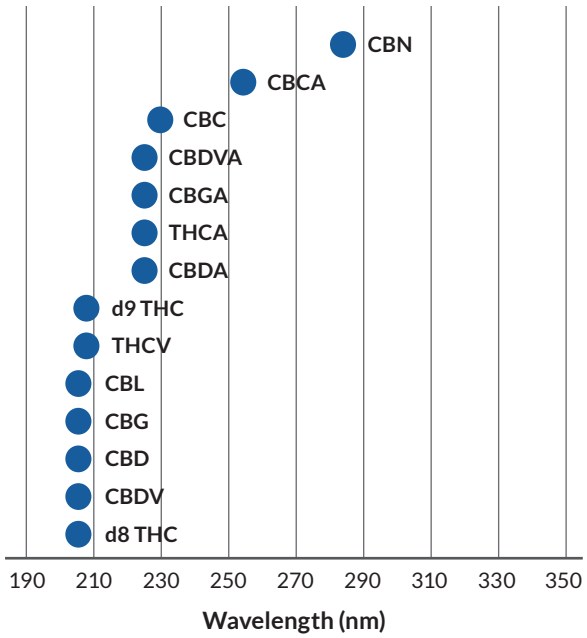
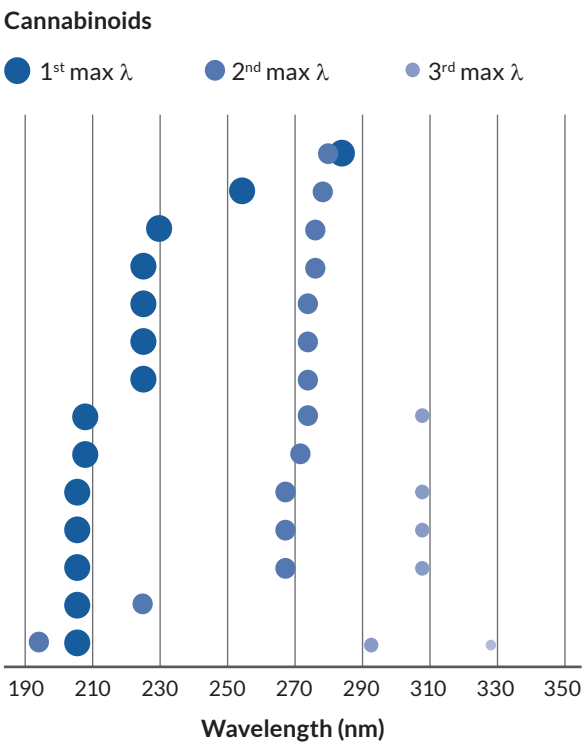


Figure 11: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

UV landscape





UV landscape

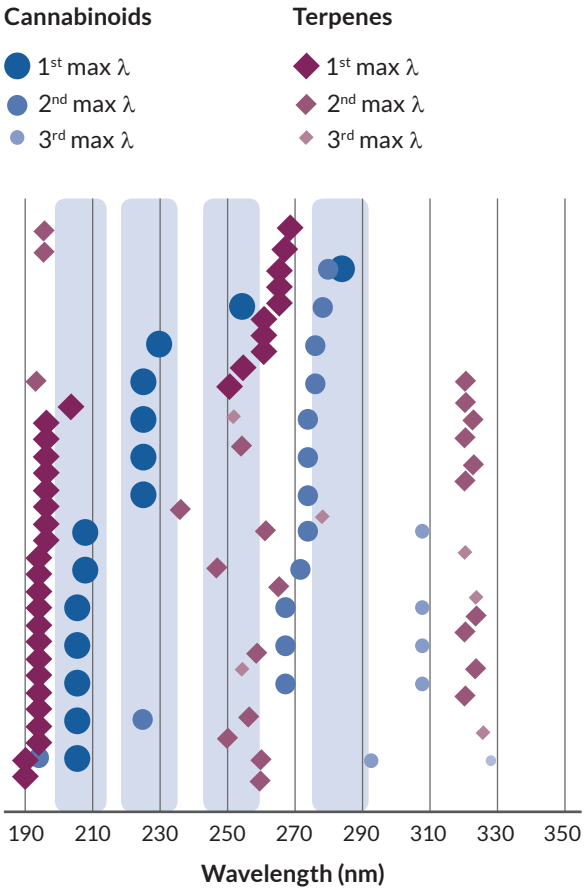


Figure 12: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

This information can also be plotted for the terpenes (pink and purple diamond shapes in figure 12C). This tells me if there are any opportunities where a signal for the cannabinoids can be, that doesn't have potential interferences. In the example here, if we look at the 230 range in figure 12. I can find out what compound is, where does it elute in my chromatogram? And is it going to cause me any issues?

So, this graphical display of the different overlapping interferences at the varying wavelengths (here we have shown four) helps us easily identify where problems will be and what the compounds are that are causing them. So, for example, here are four.

When looking at CDCA, which has a first maximum wavelength similar to where a lot of terpenes sit, and so this allows me to clearly identify chromatographically where CDCA elutes. Where the group of terpenes elute can be checked and it can be evaluated whether they will cause any issues. Figure 13 shows an example of two compounds, and you can check in terms of their UV spectra any potential issues and which wavelength to select. If they coelute, which unfortunately they do in these systems, it must be considered.

Following this we decided to look at 26 samples, 17 concentrates (non-flower) and 9 flower samples, and we knew that some of the concentrates had added terpenes in them. An outline of our method can be seen in figure 13. For calibration LC, diode array (LC-DAD), eight levels, one to five hundred ppm.

This is somewhat analyte specific, therefore in sample dilution, sometimes has to be variable just to be in the range, because the range is very wide and can be from 0.1% by weight to potentially 100% weight.

LC-MS/MS have eight levels. Essentially, we're using a ppb range and we're additional dilutions are carried out, this was even more challenging to get samples on scale. The linear dynamic range of MS is not as good, so there's also quite a bit of playing around that you have to do.

Calibration

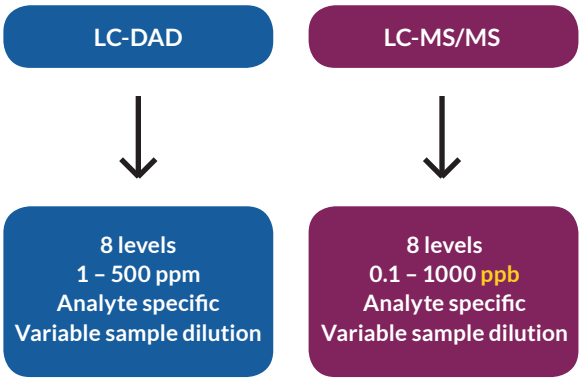
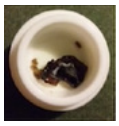


Figure 13: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

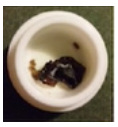


% weight

Table 1 is a summary of what we found when we compared a few different samples, and we report out our results in percent weight, this meant that everything was back calculated. The first data set was done using our current method that we're running LC-UV, and takes 17 minutes. In order to carry out this method, we looked at two different wavelengths here, which makes sense and are very close to what's in the USP monograph. And then you can also see our LC-MS/MS data in the table, where we have two different concentrates. For delta-9 THC, we get a really good agreement and I want you to note that these numbers are in the 20%, so that's what I call for a concentrate of lowish to moderate level.

Compound	Concentrate A				Concentrate B			
	LC-UV	LC-DAD		LC-MS/MS	LC-UV	LC-DAD		LC-MS/MS
	228nm	228nm	274nm		228nm	228nm	274nm	
CBC	1.8	-	2.2	1.7	2.1	2.4	2.3	2.2
CBD	22	26	24	23	28	31	29	30
CBDV	-	-	-	0.3	-	-	-	0.3
D9-THC	22	24	24	20	11	11	12	11
THCV	-	15	-	0.2	-	12	-	0.2
CBG	1.0	-	-	1.1	1.0	-	-	1.2
CBN	0.2	-	0.2	0.3	-	-	-	0.2
D8-THC	-	-	-	-	-	-	-	-
CBL	-	-	-	-	-	-	-	-
THCA	4.3	3.7	3.6	3.2	5.3	4.2	4.1	4.1
CBDA	17	16	16	13	22	20	19	19
CBGA	-	-	-	0.2	-	-	-	0.3
CBDVA	-	-	0.7	0.5	-	-	0.4	0.3
CBCA	-	-	-	0.7	-	-	-	0.5

Table 1: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*



% weight

Compound	Concentrate C				Concentrate D			
	LC-UV	LC-DAD		LC-MS/MS	LC-UV	LC-DAD		LC-MS/MS
	228nm	228nm	274nm		228nm	228nm	274nm	
CBC	0.8	1.1	1.3	1.3	0.4	-	-	0.3
CBD	-	-	-	-	-	-	-	-
CBDV	-	-	-	-	-	-	-	-
D9-THC	70	77	76	76	72	73	74	56
THCV	-	12	-	-	-	10	-	0.2
CBG	1.4	2.0	-	-	0.6	-	-	0.5
CBN	0.4	1.1	1.0	1.0	-	-	0.5	0.5
D8-THC	-	-	-	-	-	-	-	-
CBL	-	-	-	-	-	-	-	-
THCA	0.7	-	-	-	7.7	6.3	6.0	5.3
CBDA	-	-	-	-	-	-	-	-
CBGA	0.4	-	0.1	0.1	0.4	-	0.3	0.3
CBDVA	-	-	-	-	-	9.1	-	-
CBCA	-	-	-	-	-	-	-	0.4

Table 2: *Taken from Julie Kowalski's slide presentation "Reassessing Potency Analysis" at Pittcon 2018.*

Table 1 and 2 you can see that there is an issue with the compound CBD. This is why when we test for 11 but we only report 10, we know that there is an issue with it and so we don't report it but we simply monitor it.

Table 2 shows two different concentrates, which has another issue, and are concentrates with high THC, and you'll see that there's a little bit of a low bias for MS/MS. We don't know exactly what this is exactly yet. It could be multiple things. It could be the fact that we're legitimately seeing signal from other compounds in the sample that we're unaware of. It can also be, and potentially more likely, that calibration and the levels we were using for LC-MS/MS, we may have been in a region where it wasn't quite linear, or there's the potential that there was detector saturation that we weren't aware of.

05

ADVANCING ENVIRONMENTAL CHEMISTRY WITH ANALYTICAL TECHNOLOGIES

INTRODUCTION

Contamination and pollution of our air, soil, and water with chemical species resulting from human activities are threats to human health and the environment. Identifying and quantifying substances present in natural environments can give us an insight into the behavior of industry and society, aiding regulatory policy development and enforcement.

Environmental analysis is vital to identify chemical contaminants and monitor their journey though, and effects on, the environment. Pittcon 2018 featured a symposium on environmental analysis that will discuss the latest analytical techniques and their application to environmental analysis.

A recent report commissioned by the Lancet on pollution and health found that in 2015 environmental pollution resulted in the premature deaths of nine million people, accounting for 16% of global deaths, and costing trillions of dollars. Environmental pollution is a serious global problem and governments and regulatory bodies around the world are constantly setting up new regulations and targets to try to stem the flow of pollutants and contaminants into our air, water and soil.

Due to increasing global concern regarding pollution, environmental analysis is a rapidly growing, dynamic area of science which is vital for the monitoring of pollution, wastewater, drinking water, and potentially hazardous waste streams.

However, environmental analysis is a particularly challenging field as the species present in natural environments vary widely, can be completely unknown, present in low concentrations, difficult to separate from naturally occurring species, or naturally occurring species themselves.

Environmental analysis relies heavily on advanced techniques borrowed from analytical chemistry to study the identity, sources, and fates of chemical and pollutant species. Molecular spectroscopy, atomic spectroscopy, chromatography, mass spectroscopy, electroanalytical methods, thermal methods, and radiochemical methods all find applications in environmental analysis. Adequate methods of environmental analysis are vital to ensure that regulations and targets regarding water treatment, waste disposal, and air emissions are met.

5.1 ASSESSING WASTEWATER SAFETY

Wastewater epidemiology involves measuring metabolites, chemicals and/or biomarkers in sewage wastewater to obtain information about a population including population size, behavior, health, and drug consumption.

The Pittcon 2018 environmental analysis symposium featured a talk by Kevin Bisceglia on wastewater epidemiology, while the Pittcon expo will feature all the major suppliers of analytical equipment required for wastewater epidemiology.

Wastewater Epidemiology for Population Analysis

Chemicals that humans consume including medicines, alcohol, caffeine, and illegal drugs are broken down into metabolites, which are removed from the bloodstream by the kidneys and excreted as urine.

The body also produces a number of biomarkers, naturally occurring compounds that are excreted in the same way, that are indicative of the body's current state.

Measuring biomarkers present in urine can give us information about the body and its function. For instance, pregnancy tests detect the presence of hormones in the urine which are characteristic of a pregnant state. Urine tests are also routinely used to detect performance-enhancing drug use in athletes and illegal drug use in the general population.
Metabolites and biomarkers which are excreted from our bodies in urine make their way through the sewage system in wastewater, where their detection can give us valuable information about a population. Wastewater epidemiology has been used to track legal drug use, illicit drug use, population size, behaviors

(alcohol use, tobacco use, caffeine consumption, etc.), and health. Wastewater epidemiology can also be used to track drug compliance by comparing the number of patients that have been prescribed a certain drug with the number of patients that are measured to be taking the drug and excreting its metabolites.

Wastewater epidemiology involves three steps. First, raw sewage is collected and analyzed for selected substances. Secondly, concentrations of target residues are compared with the average flow of sewage and divided by the number of people the sewage treatment plant serves, yielding an average excretion rate of a metabolite or biomarker per person, and population drug loads. Functional data analysis can then provide information about geographical and temporal trends in drug consumption.

Wastewater epidemiology provides unbiased, aggregated information about a population. However, there are disadvantages to the technique. The population that is contributing to the sewage can be uncertain, metabolism and excretions can vary between individuals, and it can be difficult to differentiate anthropogenic biomarkers and metabolites from those excreted by other animals.

Best practice protocols from the Sewage Analysis CORe group Europe (SCORE) network are used to reduce uncertainties and increase the credibility of wastewater epidemiology. However, more research is still needed to improve methodologies and integrate methods from drug epidemiology, allowing more legal and illegal drugs to be identified and monitored in this way.

Jeanette van Emon from the EPA, organizer of – Assessing Community-Wide Health Via Sewage Wastewater – symposia, gave a presentation at Pittcon 2018 on – An Immunoassay for Measuring 8-Isoprostane in Sewage Wastewater to Gauge Community-wide Health.

Monitoring Illicit Drug Consumption

Monitoring illicit drug use in a population can be particularly challenging as those who take illegal drugs often prefer not to disclose their activities.

Detecting the metabolites of illicit drugs in sewage using wastewater epidemiology provides a non-invasive, unbiased way to identify the spectrum of drugs that are consumed by a population and provide geographic and temporal information about illegal drug use.

For example, a study in Australia found that cocaine and MDMA consumption were more common in urban areas compared to rural areas, while methamphetamine consumption was similar in both areas. Such information can be used to assist governments in developing policies to reduce illegal drug use.

Wastewater epidemiology has been applied globally to monitor the use of cocaine, cannabis, amphetamine, methamphetamine, and MDMA.

Analyzing Biomarkers and Metabolites in Wastewater

Detecting metabolites and biomarkers requires analysis techniques that can accurately detect and measure molecules that may be present in trace amounts in complex solutions.

Liquid chromatography (LC) and GC are often utilized in combination with mass spectroscopy to identify and quantify target compounds. Care must be taken to develop unbiased and accurate analytical procedures as metabolite and biomarker concentrations can be heavily dependent on environmental factors, sample preparation, and analysis methods. As a result, concentrations of target molecules can often be significantly underestimated.

For example, research aiming to accurately measure the concentration of the main human urinary metabolite of cannabis in wastewater found that filtration and pH adjustment during sample preparation affected the measured concentration of the metabolite and the calculated drug load.

The environmental analysis symposium at Pittcon 2018 will feature a talk by Kevin Bisceglia of Hofstra University on the use of GC-MS in wastewater epidemiology for illicit drug monitoring, and compare factors such as cost, time, robustness and environmental impact with LC methods.

Furthermore, the Pittcon Expo featured a number of leading companies offering the latest technology in LC-MS and GC-MS for wastewater epidemiology including Bruker, Shimadzu, Peak Scientific, Waters, and Metrohm. Pittcon 2018 is the ideal place to learn more about the potential of wastewater epidemiology and the analytical techniques required to analyze sewage wastewater accurately.

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5.1a DISINFECTION BY-PRODUCTS (DBPS) FROM WATER TREATMENT

Swimming pools, drinking water, and wastewater are commonly treated with disinfectants to remove harmful pathogens.

However, disinfectant by-products (DBPs) have recently been recognized as 'contaminants of emerging environmental concern' due to their potential detrimental effects on human health.

Disinfection of drinking water, wastewater, and swimming pools kills bacteria, viruses, protozoa, and algae, which may cause harm to human health. Chlorine, chloramines, chlorine dioxide, and ozone are commonly used in water treatment. However, water treatment with disinfectants can form by-products known as DBPs, many of which are considered toxic. Chlorination of drinking water, wastewater, and swimming water must be optimized to strike a balance between the risk of exposure to pathogens in untreated water and the risk from DBPs in treated water. In the US, the safe drinking water act regulates the presence of pathogens and chemical contaminants including disinfectants and some DBPs in drinking water.

What are Disinfectant By-Products (DBPs)?

DBPs form when disinfectants react with inorganic and organic matter, which is naturally present in the untreated water.

As the DBPs formed depends on the disinfectant used, which substances are present in the untreated water, and the conditions during disinfection (such as pH, temperature and disinfectant doset), there is a vast number of potential DBPs. Over 600 different compounds have been identified as DBPs so far, but not all DBP's are known, and many are not yet well characterized.

In general, disinfection with chlorine produces trihalomethanes (chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform), haloacetic acids, and chlorates; chloramine treatment produces chlorites; chlorine oxide treatment produces chlorites and chlorates; and treatment with ozone generates bromates.

Disinfectant by-products are toxic and hazardous to human health. DBPs first became a concern in 1974 when DBPs including chloroform and other carcinogens were found in chlorine-treated natural waters. DBPs can enter the human body via inhalation, skin contact, or ingestion, and they have been found in the blood and breath of swimmers and non-swimmers at indoor swimming pools.

Excessive exposure to trihalomethanes has been linked to liver, kidney, and central nervous system problems, and an increased risk of cancer. Haloacetic acids and bromate have also been linked to an increased risk of cancer, while chlorite exposure may cause central nervous system problems and anemia. Chlorate consumption has been linked to a reduced ability for red blood cells to carry oxygen and kidney failure.

The specific health risks are only known for a few DBPs. Recently discovered iodine containing DBP's are considered the most hazardous to health, followed by bromine-containing DBPs. Chlorine-containing DBPs are considered the least hazardous.

Optimizing Water Treatment

Identifying pathogens and determining the concentrations of organic compounds present in untreated water can enable authorities to make informed decisions about the correct level of water treatment.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has recently revolutionized practices for pathogen identification in water. Systems like the MALDI-Biotyper from Bruker, which were present at the 2018 Pittcon Expo, can rapidly identify a broad range of bacteria, providing vital information about the level of water treatment required.

To fully understand the formation of DBPs and make informed water-treatment decisions, it is also essential to monitor the naturally occurring organic compounds in untreated water. The MAX300-AIR™ Environmental Gas Analyzer from Extrel, who were present at the 2018 Pittcon Expo, provides continual measurement of organic compounds in water streams, enabling informed water treatment practices.

Regulating, Analyzing and Monitoring DBPs

In the US, the Environmental Protection Agency (EPA) regulates some DBPs under the clean water act. Total trihalomethane concentrations in community water systems must be below 80 ppb (µg/L), the total concentration of five haloacetic acids must be below 60 ppb, chlorite levels must be below 1 ppm, and bromate levels must be below 10 ppb. The EPA does not currently regulate chlorate.

The EPA and the International Organization for Standardization (ISO) have developed a number of analytical methods for the quantification of some DBPs in drinking water. As DBPs cover a large number of different molecules, different analysis methods are required for their quantification. There are approved analytical methods for the quantification of total trihalomethane concentrations, haloacetic acids, bromates, chlorite, and chlorate.

The approved analytical methods use GC, IC (ion chromatography), ICP (inductively coupled plasma), and colorimetric methods to determine DBP concentrations. The 2018 Pittcon Expo featured a number of leading companies supplying all the technology required for DBP analysis, including Thermo Fisher Scientific, Bruker, Extrel, and Hiden Analytical.

Identifying and Quantifying Unknown DBPs

As the prevalence and identity of all DBPs and their effects are not yet known, data collection on DBP occurrence, frequency and health effects is vital. To assess the formation, identity, and prevalence of DBPs, robust and sensitive analytical methods are required. Monitoring DBPs should be straightforward so that safe drinking water can be guaranteed. However, the vast number of potential DBPs, combined with their low concentrations can make analysis challenging.

Methods including GC-ECD (electron capture detector), GC-MS and LC-MS are commonly utilized to Identify and quantify unknown DBPs. A recent study of two Egyptian drinking water treatment plants quantified DBPs found in the raw and treated water from the plants using GC-ECD. At the 2018 Pittcon environmental analysis symposium, Tarek Manasfi of Aix-Marseille University gave a presentation on the analysis of DBPs in seawater swimming pools using GC-ECD, GC-MS and LC coupled to high-resolution MS.

The identification of iodinated DBP, which can be the most harmful to human health, can be particularly challenging due to their low concentrations. High resolution, high accuracy and high sensitivity analytical techniques are required to identify and quantify iodinated DBPs. GC coupled with high- resolution, accurate mass Orbitrap mass spectrometer, has been used to detect iodinated DBPs detection in water samples. Thermo Fischer Scientific, who will be featured at the 2018 Pittcon Expo, supply the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, which can successfully identify unknown DBPs including iodinated DBPs.

Disinfection of drinking water has reduced the presence of pathogens present in water to such a level that food now surpasses water as a leading pathogen exposure source in the US. Prepared vegetables and salads are often washed with chlorine treated water to kill any pathogens present on the leaves.

However, as the chlorine comes into contact with the organic matter of the leaves and any remaining organic matter from the soil, the risk of DBP formation is significant. William Mitch of Stanford University will give a talk at the 2018 Pittcon environmental analysis symposium on detecting DBPs in bagged lettuce and spinach using LC-MS.

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5.2 DETERMINING THE COMPOSITION OF E-CIGARETTE LIQUIDS AND SMOKE

E-cigarettes have been marketed as healthier alternatives to cigarettes, however the exact compositions of the aerosols produced by e-cigarettes and their long-term health effects are largely unknown. A range of analytical methods are required to determine the chemical exposure caused by e-cigarettes and their potential risks.

The global e-cigarette market is growing rapidly and is expected to reach a value of $27 million by 2022. Awareness of the detrimental health effects of smoking and advances in electronic device technology have driven the rapid uptake of e-cigarettes. Consumers are now widely aware of e-cigarettes and it has been estimated that 2.6-10% of adults in the US use e-cigarettes.

E-cigarettes have been marketed as healthier alternatives to cigarettes as they are tar-free, but the health risks associated with their use remain largely unknown.

E-cigarettes are battery powered, handheld devices that give the user the feel of smoking a cigarette by heating a liquid, known in the industry as an e-liquid, to generate an aerosol. The aerosol is inhaled and enters the user's mouth and lungs before it is exhaled into the environment. The e-liquid commonly consists of nicotine, propylene glycol, glycerine, and flavorings, though the exact compositions of e-liquids varies widely. Devices are often sold with defined nicotine contents from 0 to 100 mg/mL. Manufacturers often do not release information on the exact chemicals used in e-cigarette manufacture or those that may be formed when the aerosol is produced. The composition of the aerosol inhaled by the user and exhaled into the environment is often unknown.

In August 2016, the FDA introduced regulations covering the marketing, labeling, and manufacture of e-cigarettes and e-liquids. The unknown nature of e-cigarettes caused significant debate regarding their regulation. As there is no long-term data regarding the safety of e-cigarettes, debates regarding their safety and regulation have been based on scant composition analysis data and exposure estimates.

The Chemical Composition of E-Liquids and Aerosols

To understand the exposure caused by e-cigarettes, it is important to know the e-liquid composition, aerosol composition, the efficiency and consistency of the e-cigarette, and the environmental emissions. One recently analyzed e-liquid contained 64 compounds, approximately half of which were unidentified. The aerosols of the e-cigarette contained even more compounds (82); the additional compounds were volatile organic compounds formed during the heating of the e-liquid to form the aerosol.

In 2014, the FDA's Center for Drug Evaluation, Division of Pharmaceutical Analysis (DPA) analyzed e-cigarette cartridges for nicotine content and the presence of other tobacco constituents. Their testing found that the e-cigarettes tested contained detectable levels of carcinogens and other toxic chemicals.

A recent review of studies evaluating the chemicals in refill solutions, cartridges, aerosols, and the environmental emissions of e-cigarettes found that nicotine, tobacco-specific nitrosamines, aldehydes, metals, volatile organic compounds (including propylene glycol), phenolic compounds, polycyclic aromatic hydrocarbons, flavors, solvent carriers, and tobacco alkaloids were commonly present in e-cigarettes, aerosols, and/or environmental emissions resulting from e-cigarettes. Ultrafine particles with varying particle size distributions have also been reported in e-cigarette aerosols and emissions.

The exact compositions of e-liquids, aerosols, and emissions varies greatly throughout the market, and the nicotine levels listed on products are often incorrect. In addition to this, e-cigarette brands and models also vary in their efficiency and consistency of nicotine delivery.

The DPA tests revealed that the majority of e-cigarette cartridges that were labeled as nicotine-free contained low levels of nicotine.

Nicotine is an addictive and toxic chemical. Large doses of nicotine can be lethal, and even small doses are habit forming, so it important that the nicotine delivered by e-cigarettes is consistent and below toxic levels.

Furthermore, when different e-cigarette cartridges with the same label were tested, each cartridge emitted a different amount of nicotine with each inhalation, ranging from 26.8 to 43.2 mcg nicotine per 100 mL inhalation. The results of the DPA and other research suggest that the quality control processes used in e-cigarette manufacturing are inconsistent or non-existent, which could cause harm to the consumer by exposing them to increased levels of nicotine and other chemicals.

Analyzing the Composition of E-Cigarettes

A range of analytical methods can be used to determine the compositions of e-liquids, aerosols, and environmental emissions.

Techniques combining separation and mass spectroscopy, such as GC-MS and LC-MS are commonly used to identify compounds in e-liquids and aerosols. GC-MS and LC-MS provide sensitive compositional analysis.

Low-temperature plasma ionization-MS (LTPI) and electrospray ionization (ESI-MS) can also be used to detect species present in e-liquids and aerosols. ESI-MS is able to detect a variety of higher mass, less volatile species that are not observed by GC-MS. The 2018 Pittcon Expo featured all the latest technology in GC-MS, LC-MS, LTPI-MS, and ESI-MS from leading manufacturers including Thermo Fisher Scientific, Shimadzu, and Conquer Scientific.

GC-MS and LC-MS analysis of e-liquids can be labor-intensive and time-consuming. Nuclear magnetic resonance (NMR) spectroscopy has been reported to enable rapid detection of the ingredients in e-cigarette liquids. However, NMR cannot detect trace concentrations and has therefore not been able to detect tobacco-specific impurities in e-liquids. The 2018 Pittcon Expo saw Bruker, who supply automated devices and software that allows for the full automation of the NMR workflow, from sample preparation and sample changing to data analysis and archive, allowing researchers to rapidly and efficiently analyze the contents of e-cigarettes.

At the 2018 Pittcon environmental analysis symposium, John Richie from Penn State University will discuss the use of electron paramagnetic resonance spectroscopy to detect reactive, short-lived free radical species formed in e-cigarette aerosols.

To analyze the aerosols formed by e-cigarettes and the chemical exposure of the users, researchers must generate an aerosol using

the e-cigarette. As John Richie will cover in his presentation, the aerosol composition is often temperature and wattage dependent, so it is important to simulate e-cigarette usage conditions as closely as possible. Methodology for e-cigarette aerosol generation is not yet standardized and a range of equipment and parameters have been reported in the literature. Standard methods would be helpful for assessing the contents of e-cigarettes and their potential toxicity.

Investigating the Effect of E-Cigarettes on Health

The long-term health effects of e-cigarette use are not expected to be fully understood for decades. Debates on the safety of e-cigarettes have been based on the effects of chemicals found in e-liquids and aerosols. For example, tobacco-specific nitrosamines are carcinogenic compounds, and propylene glycol causes respiratory irritation.

In vitro assessments of the impacts of e-cigarettes could offer vital information about their potential long-term health effects. In vitro tests have been used previously to assess the biological impact of tobacco smoke.
In vitro aerosol exposure systems use a smoking machine to generate, dilute and deliver aerosols from the e-cigarette to cell cultures, tissues or organs Various smoking machines can be used for in vitro experiments.

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5.2a FREE RADICAL FORMATION IN ELECTRONIC CIGARETTE AEROSOLS

In this interview conducted at Pittcon 2018, John Richie discusses how free radicals in the aerosols in e-cigarettes are detected and how its impacting the environment:

What have been the main challenges associated with characterizing the harmful by-products EC can produce?

Firstly, one of the main challenges is the fact that they're so new and they've exploded onto the market. Secondly, and perhaps most importantly, is the fact that there are so many different types of products both in terms of the e-cigarettes themselves and the liquids that you fill them with.

Flavoring chemicals are a common component in e-liquids. Was there any difference in free radical production based on the flavouring chemicals used?

Flavors seem to have a pretty large impact on the radicals that are produced. There are over 7,000 different flavors available on the market today. Just to make things even more complex, each of these flavorants are made up of one or many flavoring chemicals. These chemicals each have their own different properties and they can interact with one another. It's a very complex landscape.

We've been able to test between 50 and 60 different flavors so far in terms of their impacts on free radical production. We find that many of these flavors are associated with enhanced or higher levels of free radical production. However, there are some that are associated with lower levels of free radical production. We think that might have something to do with some anti-oxidant properties of some flavors.

What characterization methods do you use to measure these free radicals?

One of the main problems with measuring these free radicals in e-cigarettes is that they're very short lived, which makes them very hard to measure. They've very reactive, which probably leads to one of the reasons why they're bad for you from a biologic perspective. What we have to do is trap them. We use spin traps, which are chemicals that will trap these free radicals and

turn them into longer-lived free radicals. We can measure these longer-lived radicals using an instrument called an electron paramagnetic resonance spectrometer or EPR, which is the only method that you can measure free radicals with directly.

What have your findings suggested and what are the potential impacts for human health?

That's the big question. We know that these are highly reactive radicals, but we don't know their chemical identity yet. We're working on that and we're close to it, but we can't ascribe specific chemical structures to them at this point in time. That will help in terms of trying to figure out exactly how toxic they may be. We have done some experiments where we have exposed biologically relevant chemicals, lipids, to these free radicals that were produced from e-cigarettes.

"We found that these free radicals oxidized these lipids in a predictable manner. We believe that these free radicals are damaging from a biological perspective. We don't yet know exactly how damaging they are in a real cell."

Dr. John Richie

Can electronic cigarettes be designed to minimize exposure to these potentially harmful products?

Our work is aiming to measure the levels of radical's produced by e-cigarettes under real conditions. We want to use this data to develop products that don't produce these toxicants.

In the case of free radicals, for example, we know that there are some flavorants that, when added to the e-liquid, can reduce the levels of free radicals. I think that alone suggests that there may be promise in terms of designing an e-liquid and an e-cigarette device that may produce negligible levels of radicals.

What are the next steps in your research?

The next step is to map out the chemical identity of these radicals. That will allow us to go a long way in terms of predicting how toxic they may be. Secondly, I think it'll be important to understand the toxicity of these free radicals in biological systems. That will involve exposing cells to e-cigarette aerosols and determining the specific effects of these radicals on important cell functions.

Why is Pittcon important for sharing your research?

I think the importance of this group stems from their expertise in measurements. E-cigarettes create a challenge in terms of measuring the radicals that are being produced from them. I think some of the techniques, some of the expertise here at Pittcon can be greatly used in terms of trying to tease apart what these e-cigarettes are producing. I'm hoping that some of the people in the audience at my talk had some neat ideas in terms of trying to see what's happening with these new and ever-changing devices.

CONCLUSION

Environmental analysis is a challenging area of analytical chemistry that aims to identify and monitor the effects of chemical contaminants on human health and the environment. Pittcon is the ideal place to learn about the latest trends in environmental analysis.

Environmental analysis relies heavily on advanced techniques from analytical chemistry including molecular spectroscopy, atomic spectroscopy, chromatography, mass spectroscopy, electroanalytical methods, thermal methods, and radiochemical methods. The Pittcon 2018 Expo featured all the leading technology suppliers and is the ideal place to find companies providing the latest analytical technology for environmental analysis.