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High-resolution Orbitrap MS for detecting new psychoactive drugs, a user's perspective

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Introduced by
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Introduction

Hello, I'm Naomi Diaz, Americas Regional Marketing Manager for Mass Spectrometry at Thermo Fisher Scientific.

Welcome to today's talk, "High-Resolution Orbitrap MS for Detecting New Psychoactive Drugs, A User's Perspective" by Dr. Marta Concheiro.

The emergence of novel psychoactive substances is an ongoing challenge for forensic toxicologists. Different analogs are continuously introduced in the market to circumvent legislation and to enhance their pharmacological activity. At the National Institute on Drug Abuse (NIDA), Dr. Concheiro's group developed two analytical methods for the determination of new psychoactive stimulants in urine by liquid chromatography – high-resolution mass spectrometry (HRAM-MS) with Thermo Scientific™ Orbitrap™ mass spectrometer technology. One method identified and quantified 28 synthetic cathinones by Target-MS/MS experiments. And in the second method, 8 piperazines, 4 designer amphetamines, and 28 synthetic cathinones and 4 metabolites were analyzed in full scan and data-dependent MS^2 mode. Both methods were sensitive (limit of quantification 0.5–5 ng/mL) and specific, fully validated, and applied to authentic cases.

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In today's webinar, Dr. Concheiro will compare these two different MS approaches to analyze new psychoactive drugs using the Thermo Scientific™ Q Exactive™ Focus mass spectrometer.

Marta Concheiro is an Assistant Professor of Forensic Toxicology at John Jay College of Criminal Justice, City University of New York. She received her Diploma M.Sc. in Pharmacy and her Ph.D. in Toxicology both from the University of Santiago de Compostela, Spain. She was a Postdoctoral Research Fellow and Research Scientist at the Section of Chemistry and Drug Metabolism at the National Institute on Drug Abuse (NIDA), NIH, in Baltimore, MD. Dr. Concheiro has more than 70 publications in peer-reviewed journals, and she has presented her work at more than 25 professional toxicology meetings. Currently her research is focused on the study of alternative matrices in forensic and clinical toxicology (oral fluid, hair, umbilical cord) and in the investigation of the novel psychoactive substances. Welcome Marta.

Presentation

Thank you so much Naomi. So today, as you've said, I'm going to talk about the high-resolution Orbitrap mass spectrometer for detecting new psychoactive substances. The new psychoactive substances are "legal" alternatives to illicit drugs that are synthesized to circumvent the current drug regulations and/or to enhance their pharmacological activity. It's a group of growing popularity because all of them are easily available through the internet, the prices are low, and the purity is high compared to the classic street drugs. The recreational use of these substances has resulted in acute and even fatal toxicities.

Novel psychoactive substances, or NPS, is a very heterogeneous group of compounds, that can be categorized based on their chemical structure or the psychotropic effects. Among the different types of drugs, the most prevalent groups are the synthetic cannabinoids, the synthetic cathinones and the piperazines.

NPS constitute a true challenge in any forensic toxicology lab. The main reason is that their number is constantly increasing; every week new compounds are showing up in the market. Because of that, we lack reference materials for many of them, which is absolutely necessary in the development of confirmation methods. Also, we don't know the pharmacokinetics of most of these drugs. So then we don't know what are the target analytes in a specific biological matrix, for example we don't know if we're still going to be able to see the parent drug in urine, or if only the metabolites are going to be present. Also, the concentration ranges that are expected are unknown.

When we develop an analytical method for NPS, this method has to have the following characteristics: it has to be sensitive and specific; it has to be easy for the incorporation of new drugs and metabolites, because it's an issue that is going to happen; and also, it's recommended to have the possibility of doing retrospective data re-interrogation, in a way that with information that we have now we can re-analyze data from samples that were injected in the past.

Nowadays, the most common analytical tools in any forensic toxicology lab are GC-MS and LC-MS/MS. In the GC-MS, we have a single quadrupole that can work in SIM mode, which means Single Ion Monitoring, we are monitoring a certain list of ions, or we can work in scan mode, where a range of masses is going to be analyzed. When we develop a confirmation method, SIM mode is preferred. With regard to LC-MS/MS, triple quadrupole is the most common instrument, and MRM mode is the most popular operational mode. In MRM mode, we're going to monitor two transitions per compound. Two transitions mean, that the parent drug is going to be isolated in Q1, the precursor I meant, then it's going to go through fragmentation in the collision cell, and finally it's going to be isolated, the product ions, in Q3.

In the last few years, the use of high-resolution mass spectrometers has been expanding. And now we have in the market two high-resolution mass spectrometers, the time-of-flight and the Orbitrap MS. The Orbitrap technology actually is the high-res mass spectrometer inside the Thermo Scientific™ Q Exactive™ MS, the instrument that we were using in our research. So, in the slide we can see how the Q Exactive MS looks on the inside, so besides the Orbitrap, we have a quadrupole, we have a collision cell, and we have a very important piece, the C-Trap, that is absolutely necessary for the correct functioning of the Orbitrap analyzer. So, due to this configuration, we can actually have a lot of different operational modes with this instrument, besides the SIM, scan, MRM, that we saw with the previous ones.

So, let's have a quick look. With this type of instrument, we can work in a simple full scan, in a way that the quadrupole is not working, the collision cell is not working, and we are doing a full scan in high resolution of all the precursors with the Orbitrap MS. With this kind of instrument, also we can combine different kinds of operational modes in the same experiment. For example, we can do full scan and what is called, AIF, all ion fragmentation. What that means is that all the precursors are going to go to the collision cell and then we're going to scan for all the products that are generated with the Orbitrap analyzer in high resolution. Then we have the last two modes that actually we're going to talk about more a little bit later in the presentation. The first one is the Targeted MS^2. In this case, a certain number of precursors can be isolated in the quadrupole, they are going through fragmentation in the collision cell, and finally the product ions are going to be scanned in high resolution in the Orbitrap analyzer. This Targeted MS^2 is the closest mode to the MRM mode in the triple quad. And finally, we have the combination of the full scan, as we described above, and the data dependent MS^2. So, what is the meaning of this data dependent MS^2? In this mode, when a precursor satisfies a certain selection criteria, is isolated in the quadrupole, going through fragmentation in the collision cell, and then we'll get the full scan of the product ions.

Nowadays there are many analytical methods that have been published for the detection of NPS. Many of them were developed with MRM mode, with a triple quadrupole. All these methods are sensitive and specific but they have two important limitations. First, due to the time segment restrictions, the number of transitions that can be monitored in a certain time segment, the number of compounds that can be included in the method has a limit, and also the drugs that were not included in the method cannot be detected, because we'll only see the transitions that we introduced. There are also some publications that have been done with high resolution instruments, and one approach that can be done with these instruments, as I said before, is scan and data dependent methods. All these methods showed to be specific and sensitive, they have the good characteristic that there is no time segment restriction, so we can add a very high number of compounds, and also that retrospective data interrogation is possible.

Now I'm going to discuss two methods that we developed at NIDA using the Q Exactive MS from Thermo Fisher Scientific. In the first method, our goal was to do a quantification method for 28 synthetic cathinones and metabolites in urine by liquid chromatography - high-resolution mass spectrometry. And our first goal was to develop a very sensitive and specific method. The compounds that were analyzed were 28 synthetic cathinones, that included 12 ring, amine or side chain substituted, as we can see in this figure right here, also 4 methylenedioxy-substituted compounds, and 8 pyrrolidinyl-substituted. And on top of that we also included 4 metabolites.

The MS approach we chose with the Q Exactive mass spectrometer to get a very sensitive and specific method was the Target-MS^2. In this mode, we're going to isolate a certain number of precursors in the quadrupole. They are going to go directly to the collision cell for fragmentation, and after a step in the C-trap, these are going to be sent in the Orbitrap analyzer to obtain the information of the product ions. We're going to do a scan, because obviously the Orbitrap analyzer is always working in scan mode, so out of all the masses, we're going to extract two ions, product ions; one will be used for quantification and the second one for confirmation.

The identification criteria that we employed in this method was exactly the same identification criteria that you have to use in MRM mode with a triple quad. So, what does it mean? That to identify a peak, it has to have the right retention time, within 0.2 min of the calibrators, the presence of two product ions, and the ion ratio between these two product ions has to be within the 20% of the ion ratio of the calibrators. So just to emphasize, we can say that we didn't save any identification criterion because we were using high resolution, and because of that we were getting a very specific method.

We cannot forget that the compounds that we have to deal with, these synthetic cathinones, all of them share a very common chemical structure, and there are very small modifications among the different derivatives, and even the metabolites. Because of that, the probability that we have two compounds that have exactly the same molecular weight, and later on, exactly the same fragments, that possibility is high. So, even though we were using a high-resolution mass spectrometer, we couldn't forget about the chromatographic separation, because without this chromatographic separation in time, we won't be able to make the difference among these compounds.

The liquid chromatography was the Thermo Scientific™ UltiMate™ 3000 system and a C18 reversed phase column Accucore™ (Thermo Fisher Scientific), and the mobile phase was a combination of 0.1% formic acid in water and in acetonitrile. I just want to highlight that the slope of the gradient had to be very slow to allow the total resolution among the isobaric compounds.

The solid phase extraction of the samples was performed by mixed mode-strong cation exchange cartridges, Thermo Scientific™ SOLA™ CX, and in this extraction, we just employed 250 µL of urine at pH 6, and after washing with acetic acid, methanol, and hexane/ethylacetate, we performed a final elution with dichloromethane, isopropanol and ammonium hydroxide.

In the validation, we went through all the parameters that are specified in the SWGTOX guidelines. In the LOD/LOQ, it was determined by decreasing concentrations. In all cases it was just 0.5 ng/mL, and I just want to highlight when we see the chromatograms right here that even at these low concentrations in urine samples the noise was very low due to the high specificity that we achieved. The linearity was between 0.5 and 100 ng/mL. No matrix effect was detected, and the process efficiency was above 80%. The bias was less than 8, and imprecision less than 6. The specificity of the method, as I already said, it was high and we didn't get any interference from common drugs of abuse or endogenous components in the urine samples.

Here we can see an extracted ion chromatogram of the quantifier product ion from an authentic urine specimen that was positive for four synthetic cathinones, methylone, pentedrone, pentylone and alfa-PVP. So even though some of these synthetic cathinones were at pretty low concentration, 1.7, 3.5, again the noise was almost zero.

After developing this method, we achieved the goal of the specificity and the sensitivity; however, we observed a limitation. Because of the Target-MS^2 approach, just a certain number of compounds could be included in the method. And as I said before, NPS are evolving every week; new drugs are showing up, so we need methods that can add potentially unlimited numbers of drugs. Because of that we decided to develop another method, again with the Q Exactive MS, but with a completely different MS approach. In this case we did the simultaneous determination of 40 novel psychoactive stimulants in urine, using the Q Exactive MS and library matching.

The MS approach in this case, was the experiment full scan and data-dependent MS^2. That means, that the instrument is constantly running in full scan, so we are getting the full scan information in high resolution of all the precursors that are in the sample, and when the precursor meets the selection criteria, a product ion scan is triggered, so then we can get the product ion scan information for that specific compound, and this one through library matching will be used for confirmation.

If we see the details of the MS parameters, we have the full scan, that information is actually going to be used from the precursor for the quantification of the peak; we analyzed a range of masses between 100 and 400, and a resolution of 35,000. If the precursor that was in an inclusion list, showed an intensity above the values shown in the slide, the data dependent MS^2

was triggered. This data dependent MS^2 consisted in 1 to 2 scans at the apex of the peak, and this scan was obtained at a resolution of 17,500, and it was used for confirmation. This brings me to another important highlight of the Q Exactive MS, that depending on the experiment that you are performing, you can select different resolution of the instrument, different power, and that it will help you to optimize the speed of the instrument.

Here we see the full scan of the total ion chromatogram of urine at 2.5 µg/L or ng/mL. We see here all the compounds that are in our method and this is the full scan information. It happens that for every one of these 47 peaks, because they met the selection criteria, at the tip of the peak we obtained the product ion scan. Let's see for example the peak number 29, oops sorry, that it was the mCPP. We obtained at the tip this product ion spectrum that actually was used after matching against the library as confirmation of that peak.

We developed that library in the lab. So, we were injecting the compounds individually and obtaining the product ion spectra for all of them. We built the library with Library Manager, and then we processed all the data with Thermo Scientific™ TraceFinder™ software. Then we decided what kind of search to do, finally we decided to go for the reverse searching that means that it determines if any library spectra are included in the unknown spectrum. In the final score that we got with the matching, the product ion exact mass was also taken into account as well as the product ions relative intensities. After several experiments, we found out that a score equal or greater than 60 was highly specific and it was our cutoff.

In this method, the identification criteria as you can see, is quite different than the Target-MS^2. With this approach with the full scan and data-dependent MS^2, to identify a peak had to have the right retention time within 0.2 min compared to the calibrators, then the precursor ion exact mass had to be within 5 ppm of the theoretical mass. With the information we obtained with the full scan, and the product ion spectrum, library score had to be equal or greater than 60.

In this method, again we even had more number of compounds, we couldn't forget about working hard on the chromatography to be able to resolve the isobaric compounds. Again, we applied the same pump and the same column, and a very similar gradient.

The solid phase extraction was performed again with the cation exchange. It was the best choice, since all these new psychoactive stimulants are basic drugs. In this method, we only used 100 µL of urine that was buffered at pH 6. After washing with acetic acid and methanol, we performed the final elution with dichloromethane, isopropanol, and ammonium hydroxide.

As we did with the previous method, we went through a complete validation. The linearity was between 2.5 or 5, depending on the compound, to 500 µg/L. LODs, LOQs were between 1 and 5 µg/L. Imprecision was less than 15%, accuracy between 84 and 118%, process efficiency between 62 and 174%, and regarding the matrix effect, 38 compounds did not show matrix effect, 4 showed ion suppression and just 2 had ion enhancement.

In this slide, we can see the extracted ion chromatogram of the exact mass of the precursor that were obtained in full scan, from an authentic urine sample that was positive for three of these compounds, alfa-PPP, alfa-PVP, and MDPV. All the peaks showed a great signal-to-noise ratio as well.

In summary, I just want to say that we developed two analytical methods for the determination of NPS in urine samples. In one method, we applied the Target-MS approach, and it showed to be highly sensitive and specific; however, just a limited number of compounds could be included in the method. In the second analytical method, we applied the full scan and the data-dependent MS^2 approach. Again, the method was highly sensitive and specific, and it added two big advantages. It was very easy to add new analytes, the only thing we need to do is to obtain the product ion spectrum to be integrated in the library, and the retrospective screening analysis was possible because the precursor information was obtained in scan mode so we can always go back to analyze those data.

Finally, I just want to thank my previous PI, Dr. Marilyn Huestis, and all the staff from the Chemistry and Drug Metabolism Section at NIDA, and all of you for your attention.

Resources

1. eBook: Identify Rapidly Evolving Novel Psychoactive Drug Substances Faster.
2. Tech Note: Determination of Cannabinoids in Low Volume Human Whole Blood Samples by Automated On-line Extraction using Turbulent Flow Chromatography and High Resolution/Accurate Mass Hybrid Quadrupole Orbitrap Mass Spectrometry.
3. Concheiro M, Castaneto M, Kronstrand R, Huestis MA. Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography-high resolution mass spectrometry and library matching. *J Chromatogr A.* **2015** Jun 5;*1397*:32–42. doi: 10.1016/j.chroma.2015.04.002. Epub 2015 Apr 8.
4. Concheiro M, Lee D, Lendoirob E, Huestis MA. Simultaneous quantification of Δ9-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography–high resolution mass spectrometry. *Journal of Chromatography A,* Volume 1297, 5 July 2013, Pages 123–130.
5. Concheiro M, Anizan S, Ellefsen K, Huestis MA. Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resoltuion mass spectrometry. *Analytical and Bioanalytical Chemistry* **(2013)** *405*(29):9437–48.
6. eBook: Mass Spectrometry Workflows Solving Forensic Cases.
7. eBook: Promises and Pitfalls of Alternative Matrices: Capture the Drug Traces You Need.
8. Should LC-MS Replace Immunoassay?

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