Case Study

Ensuring Food and Dietary Supplement Safety Using High-Resolution Accurate-Mass Orbitrap-Based Mass Spectrometer Detection

"The Q Exactive mass spectrometer can screen for the adulterants we do know as well as for the ones that are unexpected, and if we find unexpected compounds, we can use the Q Exactive data to identify them."

Dr. Katerina Mastovska
Associate Scientific Director
Nutritional Chemistry and Food Safety
Covance® Laboratories

Quality, Safety and Compliance

Covance's Nutritional Chemistry and Food Safety (NCFS) group is a contract research laboratory, which provides analytical testing and method development services in the areas of nutrition, microbiology, and contaminants. Industries served include the food, infant formula, dietary supplement, animal health, and agricultural biotechnology industries. While many analytical tools are used to address the NCFS' broad menu of analytical services, the group recently selected the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer for detection and identification of contaminants and adulterants.

Analytical Challenge

Due to the diversity of sample matrices encountered and known and unknown compounds that must be detected and identified, food and supplement analyses present significant analytical challenges. Analytical methods and instrumentation must be able to reliably accommodate this diversity at a reasonable cost with high sample throughput and sensitivity. Ideally it would be possible to screen and quantitate hundreds of target compounds in a single analysis.

Adding to the challenge, the number of known and unknown compounds grows all the time. For example, new adulterants that are modified structures of known drugs are continually introduced. Thus it would be desirable to detect any potential contaminant in any matrix. In fact, this is where Katerina Mastovska, Ph.D., Associate Scientific Director at Covance, believes contaminant testing is headed: from highly targeted to non-targeted methods that are able to provide much more comprehensive information about a sample.

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▲ NCFS scientist, Dr. Lukas Vaclavik, performs an analysis on a Q Exactive Plus mass spectrometer in a Covance laboratory in Harrogate, UK.

The Solution

To address these challenges, Dr. Mastovska and her team at Covance NCFS performed a hands-on, in-lab evaluation of several vendors' MS systems. They selected the Q Exactive mass spectrometer due to its ability to acquire data in both a targeted and non-targeted fashion, with a very high mass resolution and accuracy, in both MS and MS/MS modes.

With this unique capability, the NCFS can now develop and deploy methods able to detect and identify known and unknown compounds rapidly, in one analytical run. The mass accuracy obtained for both MS and MS/MS data significantly increases their confidence in compound identifications. Compounds are identified using high-resolution accurate-mass MS/MS spectral databases and libraries the NCSF has developed in collaboration with Thermo Fisher Scientific.

The databases allow up to 10 MS/MS fragments per compound, which provides rich information for identification of compounds including isomers. When the instrument is operated in all-ion fragmentation (AIF) mode, MS/MS fragments are measured with better than 1 ppm mass accuracy. Analyte-specific conditions and instrument settings are not needed to collect full-scan MS and MS/MS data.

High resolution and wide dynamic range enable NCFS scientists to easily distinguish between analytes and matrix, as well as between milligram-level adulterants and trace-level contaminants. Having an instrument that can do both is crucial.

Example Case: PDE5 Inhibitor Adulterants

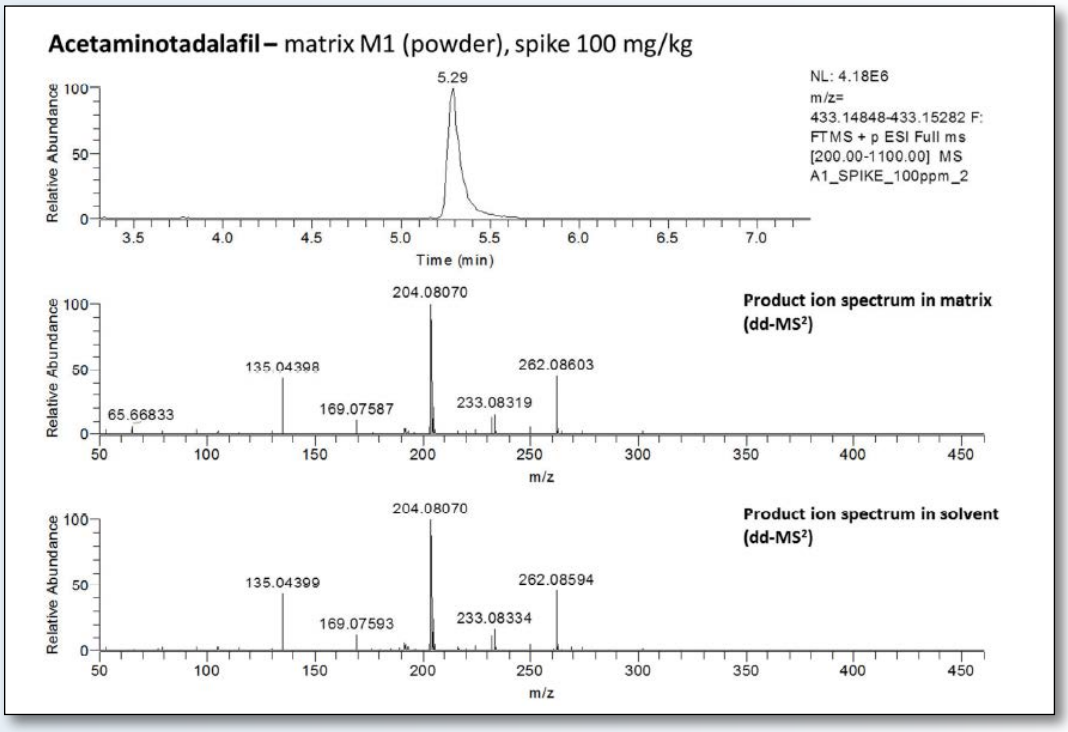
Adulteration of dietary supplements with active pharmaceutical ingredients and their analogues provides an excellent example of the challenges Dr. Mastovska and her team face and how these challenges are addressed using the Q Exactive mass spectrometer. Sexual enhancement supplements are among the most adulterated, and the adulterants are often the Phosphodiesterase type 5 (PDE5) inhibitors used by healthcare professionals to treat erectile dysfunction and pulmonary hypertension. These compounds inhibit specific enzymes allowing blood vessels to relax, which in turn increases blood flow. Adulteration of over the counter supplements is a serious problem because prescription PDE5 inhibitors have a long list of side effects and potentially harmful interactions with other prescription drugs.

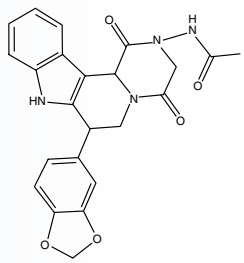
PDE5 inhibitors are heterocyclic organic compounds composed of various combinations of benzene, pyrrolidine, pyrimidine, pyrazolopyrimidine, and piperazine rings. There are approximately 150 known PDE5 inhibitor analogues (structurally similar compounds that have been modified for example with the addition or deletion of functional groups) of which AOAC maintains a list at www.eoma.aoac.org. Over 50 of these have been reported as adulterants.

Manufacturers of illegitimate PDE5 inhibitors continue to develop new analogues of legitimate PDE5 inhibitors that may be mixed together or embedded into capsules. PDE5 inhibitor testing methodology must be able to differentiate these analogs, even if they are haven't been created yet. The variety of dietary supplement matrices – tablets, capsule contents and shells, softgels, gelcaps, liquids, powders, and extracts – adds to the complexity of the analysis.

Because of its sensitivity and selectivity for analytes in complex mixtures, LC-MS is typically used for the analysis of PDE5 inhibitors and their analogues. The structure of unknown analogues is predicted by comparing the characteristic MS/MS fragmentation patterns of unknown analogues with those that are known. Ideally, to achieve the shortest time to results, the LC-MS method should be able to screen for known and unknown PDE5 inhibitors in various matrices in a single analytical run, with the ability to quantitate PDE5 inhibitors for which reference standards are available.

As a consequence of the seriousness of the problem, AOAC INTERNATIONAL is establishing voluntary consensus standards under contract with the National Institutes of Health-Office of Dietary Supplements (NIH/ODS). As chair of the working group on the PDE5 inhibitors, Dr. Mastovska was directly involved in the development of AOAC Standard Method Performance Requirements (SMPRs) for screening, identification and quantitation of PDE5 inhibitors in dietary ingredients and supplements. Based on those SPMRs, AOAC INTERNATIONAL issued a call for methods. In response to this call, Covance's NCFS scientists developed and validated a screening and identification method for PDE5 inhibitors using the Q Exactive mass spectrometer. This method was approved by an AOAC INTERNATIONAL Expert Review Panel as the AOAC First Action Official method 2015.12 for screening, identification and also quantitation of PDE5 inhibitors in dietary ingredients and supplements.





◀ Example chromatogram and product ion spectra of Acetaminotadalafil in an extract of botanical powder and solvent demonstrating high selectivity of the method and reliable identification of the analyte. Acetaminotadalafil is an analogue of Tadalafil, the PDE5 inhibitor sold under the brand names Adcirca® and Cialis®.

Conclusion

For Dr. Mastovska, Covance's NCFS method that uses the Q Exactive mass spectrometer provides an efficient solution. The method combines full-scan MS analysis with data-dependent MS/MS and AIF experiments, enabling both targeted screening and quantification of known PDE5 inhibitors and non-targeted screening and identification of novel analogues, without a need to develop analyte-specific conditions for data acquisition and analysis.

About Covance

Covance Nutritional Chemistry and Food Safety Services provides industry-leading analytical testing services to the global marketplace, including food, infant formula and dietary supplements. With locations in the Americas, Europe and Asia-Pacific, Covance scientists deliver nutritional chemistry, contaminant, microbiological testing and consulting solutions that allow their clients to mitigate risk and protect their brand.

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