Screening and Identification of Adulterants in Weight Loss Supplements by UHPLC and High-Resolution Accurate-Mass Detection

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Introduction/Abstract

The dietary supplement market segment has been growing worldwide at 5% or more year over year for an extended period of time. One particularly popular category is weight-loss products. Unfortunately, these products are sometimes adulterated with synthetic weight-loss drugs that have anorectic or laxative effects (such as sibutramine and its analogs) and also with antidepressants to suppress side-effects of these drugs. Therefore, screening and identification of a wide range of adulterants are prerequisites for many supplement distributing companies to ensure consumer safety, comply with regulations and protect their brand. We developed a high-resolution/accurate-mass screening and identification method using a Q Exactive™Plus (Thermo Fisher) instrument, which provides a wide analytical range allowing for detection of both very low (contamination) levels and also very high (adulteration) levels, which can occur in real-world samples. A combination of full scan MS-data dependent MS/MS and all ion fragmentation (AIF) was employed to acquire data for both known (targeted) and unknown (non-targeted) compounds. Figure 1 contains an overlay of approximately 50 targeted compounds. Our data processing workflow incorporated an in-lab generated database of potential weight-loss supplement adulterants to match analyte retention time, precursor mass, isotopic pattern and up to ten exact-mass fragments. Additionally, the AIF option allowed for retrospective analysis of the data and search for compounds not included in the database. A simple dilute-and-shoot sample preparation was used and evaluated in the analysis of a wide range of weight-loss supplement sample types (capsules, tablets, oils, liquids, powders and gummies) that we purchased and analyzed together with over-spiked extracts to establish sample preparation and instrument detection/identification performance.

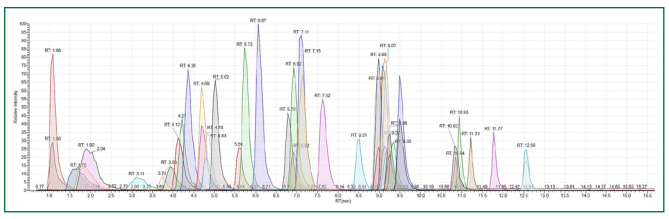

Figure 1. Overlay of approximately 50 targeted compounds.

Sample Preparation and Analysis

Numerous representative samples were obtained from various sources to cover a broad range of dietary supplement types and matrices (Table 1). Samples were prepared by portioning a single tablet, capsule, soft gel, etc. into a scintillation vial, followed by a portion of 50:50 acetonitrile in water. The tablets were ground and capsules were opened in order to speed extraction. The samples were capped and sonicated in 15-minute increments until there was no observable progress in the sample disintegration/dissolution. The extract was centrifuged and the supernatant was diluted 1:10 with 30:70 acetonitrile in water and filtered prior to the analysis.

Analysis was performed by injecting a 5 µL aliquot onto an Agilent® Zorbax® Eclipse C18 RRHD column and eluting with water and methanol containing ammonium formate and formic acid as modifiers. In addition to HR-AM detection, a diode array is also included in the UHPLC configuration. Collecting full scan (190-900 nm) aids in focusing attention to areas of interest in the chromatogram by highlighting potential adulterants containing certain chromophores.

The Q Exactive™Plus instrument was operated with the series of experiments outlined in Figure 2. Positive/negative mode switching was employed to cover the widest range of potential adulterants. For each mode, full scan MS-data dependent MS/MS (dd-MSMS) and all ion fragmentation (AIF) was employed to acquire data for target and non-target compounds. Table 2 provides a list of analytes evaluated as target compounds in this study. Precursor ions (pseudomolecular ions) for these analytes were used in the inclusion list for dd-MSMS.

The cycle time is about 1 second, and was optimized for the number of data points per peak by increasing or decreasing the default resolving power (R), adjusting the number of compounds to perform a data dependent fragmentation per cycle and selecting a single set of fragmentation energies to use for all experiments.

Table 1. Representative Dietary Supplement Samples from Various Sources

Dietary Supplement Category	Range of Ingredients
Tablet	Calcium pyruvate, pyridoxine hydrochloride, chromium, jujube, chamomile flower, acai, senna leaf, rhubarb root, licorice root, hydrangea root, artichoke leaf, selenium, copper, guarana seed, green tea leaf
Soft gel	Eicosapentaenoic acid, docosahexanoic acid, conjugated linoleic acid, γ-linolenic acid, olive oil, avocado oil
Gummy	Olive extract, cumin extract, wild mint, lady's mantle extract, goji extract
Powder	Wild olive, wild mint, komijn, citrus aurantium, paullinia cupana, cacao, green coffee bean, ashwagandha, yohimbe, L-theanine, L-carnitine, L-tartrate
Liquid	Magnesium, chromium, chloride, sulfate, boron, L-carnitine, pantothenic acid
Capsule	Yohimbine, rauwolscine, theobromine, citrus aurantium, green coffee bean, garcinia cambogia, sulbutiamine, β-phenethylamine, hordeum vulgare, fursultiamine, citrus reticulata, synephrine, yohimbe, oolong tea, isobutryl thiamine disulfide, raspberry ketone, evodiamine, ginger root

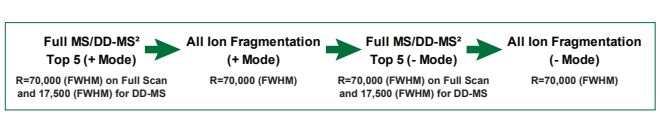

Figure 2. Series of experiments for Q Exactive™Plus.

Table 2. Analytes Evaluated as Target Compounds

Analyte Name	Chemical Formula	Adduct	Extracted Mass (Da)	Limit of Identification* (ppm)
2-Methylamino-1-phenylbutane	$C_{11}H_{17}N$	M+H	164.14338	1
Aegeline	$C_{18}H_{19}NO_3$	M+H	298.14377	10
Amphetamine	$C_9H_{13}N$	M+H	136.11208	1
Benzphetamine	$C_{17}H_{21}N$	M+H	240.17468	1
Benzylsibutramine	$C_{20}H_{26}ClN$	M+H	314.16700	1
Bumetanide	$C_{17}H_{20}N_2O_5S$	M+H	365.11657	1
Bupropion	$C_{13}H_{18}ClNO$	M+H	240.11497	1
Caffeine	$C_8H_{10}N_4O_2$	M+H	195.08765	10
Cetilistat	$C_{26}H_{39}NO_3$	M+H	402.30027	100
Chlorosibutramine	$C_{17}H_{28}Cl_2N$	M+H	314.14368	1
Dapoxetine	$C_{21}H_{23}NO$	M+H	306.18524	10
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	M+H	296.02396	1
Diethylpropion	$C_{13}H_{19}NO$	M+H	206.15394	1
Emodin	$C_{15}H_{10}O_5$	M-H	269.04555	1
Ephedrine	$C_{10}H_{15}NO$	M+H	166.12264	1
Methylephedrine	$C_{11}H_{17}NO$	M+H	180.13829	1
Methylpseudoephedrine	$C_{11}H_{17}NO$	M+H	180.13829	1
Norephedrine	$C_9H_{13}NO$	M+H	152.10699	1
Norpseudoephedrine	$C_9H_{13}NO$	M+H	152.10699	1
Pseudoephedrine	$C_{10}H_{15}NO$	M+H	166.12264	1
Fenfluramine	$C_{12}H_{16}F_3N$	M+H	232.13076	1
Fenproporex	$C_{12}H_{16}N_2$	M+H	189.13863	1
Fluoxetine	$C_{17}H_{18}F_3NO$	M+H	310.14133	1
Furosemide	$C_{12}H_{11}ClN_2O_5S$	M-H	329.00044	1
Glybenclamide	$C_{23}H_{28}ClN_3O_5S$	M+H	494.15110	1
Homosibutramine	$C_{18}H_{28}ClN$	M+H	294.19830	1
Hordenine	$C_{10}H_{15}NO$	M+H	166.12264	1
Lorcaserin	$C_{11}H_{14}ClN$	M+H	196.08875	1
Metformin	$C_4H_{11}N_5$	M+H	130.10872	1
Methylbenzylamine	$C_8H_{11}N$	M+H	122.09643	250
Methylhexanamine	$C_7H_{17}N$	M+H	116.14338	250
β-Methylphenethylamine	$C_9H_{13}N$	M+H	136.11208	1
N,α-Diethylphenethylamine	$C_{12}H_{19}N$	M+H	178.15903	1
N,N-Dimethylphenylethylamine	$C_{10}H_{15}N$	M+H	150.12773	1
N-Desmethyl sertraline	$C_{16}H_{15}Cl_2N$	M+H	292.06543	1
N-Desmethyl sibutramine	$C_{16}H_{26}ClN$	M+H	266.16700	1
N-Didesmethyl sibutramine	$C_{15}H_{22}ClN$	M+H	252.15135	1
N-Formyl-N,N-didesmethyl sibutramine	$C_{16}H_{22}ClNO$	M+H	280.14627	1
NIDA-41020	$C_{23}H_{26}Cl_2N_4O_2$	M+H	459.13491	1
N-Methyltryptamine	$C_{11}H_{14}N_2$	M+H	175.12298	1
N-Methyltyramine	$C_9H_{13}NO$	M+H	152.10699	1
Octopamine	$C_8H_{11}NO_2$	M+H	154.08626	100
Orlistat	$C_{29}H_{53}NO_5$	M+NH_4	513.42620	100
Paroxetine	$C_{19}H_{20}FNO_3$	M+H	330.15000	1
Phendimetrazine	$C_{12}H_{17}NO$	M+H	192.13829	1
Phenolphthalein	$C_{20}H_{14}O_4$	M+H	319.09649	1
Phentermine	$C_{10}H_{15}N$	M+H	150.12773	1
2-Phenylethylamine	$C_8H_{11}N$	M+H	122.09643	250
Phenytoin	$C_{15}H_{12}N_2O_2$	M+H	253.09715	10
Propranolol	$C_{16}H_{21}NO_2$	M+H	260.16451	1
Rimonabant	$C_{22}H_{21}Cl_3N_4O$	M+H	463.08537	1
Sertraline	$C_{17}H_{17}Cl_2N$	M+H	306.08108	1
Sibutramine	$C_{17}H_{26}ClN$	M+H	280.18265	1
Synephrine	$C_9H_{13}NO_2$	M+H	168.10191	1
Theobromine	$C_7H_8N_4O_2$	M+H	181.07200	10
Theophylline	$C_7H_8N_4O_2$	M+H	181.07200	10
Topiramate	$C_{12}H_{21}NO_8S$	M+NH_4	357.13261	1
Tyramine	$C_8H_{11}NO$	M+H	138.09134	10

* Limit of identification varies by matrix.

Detection and Identification

Analyte detection and identification were evaluated in sample extracts fortified (before the final dilution and filtration) with a mixture of the tested adulterants at three to four levels corresponding to 1, 10, 100, 1,000 and 2,000 ppm in sample. The analyte spiking mixture was designed based on previously estimated limits of identification for each compound. The criteria for compound detection and identification include: extraction of a peak at the expected retention time within a 30-second window with mass accuracy of +/- 5 ppm; an isotopic pattern match for the precursor ion; and one or more fragments matched from the precursor molecule with intensity above a minimum threshold. Several workflow steps are presented in Figures 3 and 4.

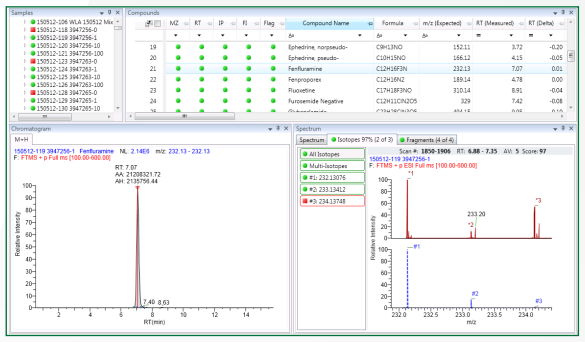

Figure 3. Workflow step for the determination of the isotopic pattern in a powder sample spiked with fenfluramine at 1ppm.

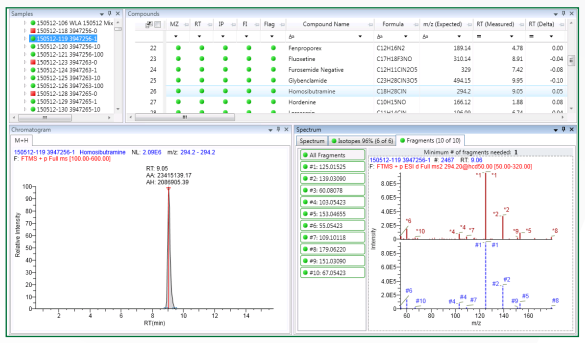

Figure 4. Workflow step for the evaluation of the fragments observed for homosibutramine in a powder sample spiked at 1ppm.

Results and Discussion

Most of the analytes were detected and identified at all spiking levels. Typical mass errors for the extracted precursors were -2 to +2 ppm, while the error observed for the fragments was higher at 2-5 ppm. This is expected as the resolution of the Q Exactive™Plus was reduced for the DD-MS² experiments. It was also noted that using our in-house developed specifications, we saw a very high detection rate of the fragments at all concentrations, typically 50% or more of the 2-10 fragments in our database for each analyte were found. Detection of the fragments is dependent on the concentration of the analyte in solution present at the source. In neat solutions we will normally match 80-100% of the fragments at a concentration equal to the Limit of Identification diluted 100X. The analytes with the lowest sensitivity were early eluting (1- and 2-phenethylamine, octopamine and synephrine) and late eluting (cetilistat and orlistat) compounds. Additional spiking experiments will be conducted at higher concentration levels in order to establish reporting limits for these analytes for routine analysis. Also, three isotopically labeled internal standards will be added into the analytical procedure as a quality control measure of the determination step. These will span the chromatographic separation from caffeine (-$^{13}C_3$, RT=5 minutes) through topirimate (-$^{13}C_6$, RT=7 minutes) and out to sibutramine (-D_6, RT=9 minutes).

The in-house generation of a database containing all of the data points necessary for the data processing allowed us to elucidate many pieces of information for the future of this type of testing. The most powerful data are the accurate mass fragments. By establishing a set of fragments for sets of analogs, we can interpret (AIF) fragmentation data, looking for common fragments originating from the same base molecule, i.e., sibutramine analogs usually contain 125.01525, 139.03090, 153.04655 and 103.05423 Da fragments. Figure 5 shows fragmentation patterns of several sibutramine analogs. Note that the benzyl- and chloro- analogs do not contain a high abundance of several of the expected fragments because of the molecule's starting structure. Figure 6 contains some analogs that belong to the phenethylamine, sibutramine and ephedrine groups to show the similarities in structure.

Many of the tested matrices had endogenous levels of certain analytes that could potentially be used as adulterants/undeclared additives. The most commonly found endogenous compounds were benzphetamine, caffeine, emodin, fenproporex, hordenine, 1- and 2-phenethylamine, β-methylphenethylamine, N-methyltryptamine, N-methyltyramine, synephrine, theobromine and theophylline.

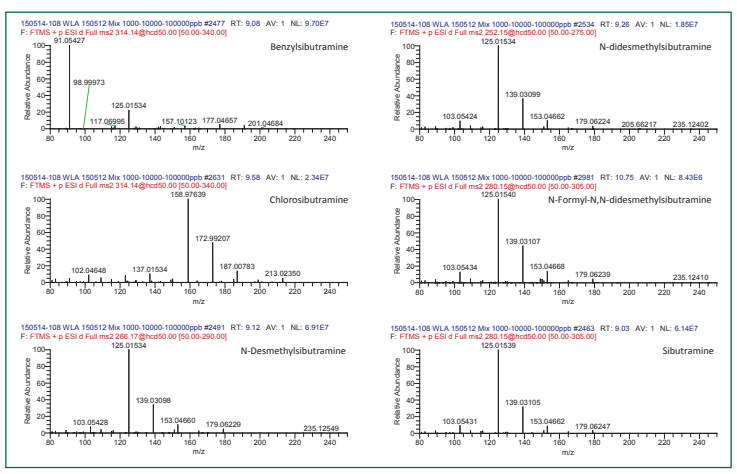

Figure 5. Fragmentation patterns of several sibutramine analogs, showing similarities and differences.

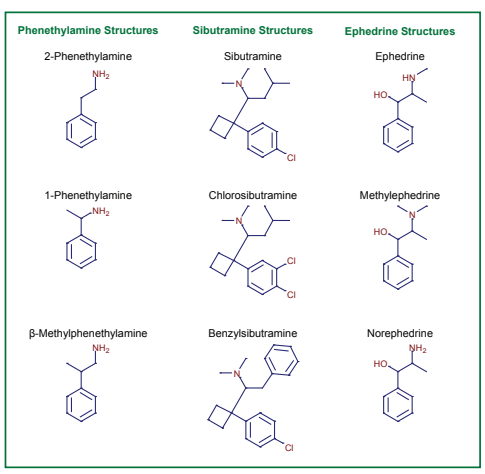

Figure 6. Similarities in structure for analogs in phenethylamine, sibutramine and ephedrine groups.

Conclusions

The current method allows for detection, identification and has the potential for quantification of more than 50 LC-MS amenable compounds that are innately present or potentially adulterated in various dietary supplement matrix types, especially those that are most commonly used in the weight-loss arena. Incorporating new software applications like Compound Discoverer, m/zCloud or Sieve® (Thermo Finnigan, LLC) will allow additional interpretation techniques for automated targeted and non-targeted screening of both known and unknown compounds.

Presented at NACRW 2015

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