Development and Validation of an Analytical Method to Identify and Quantitate Novel Modafinil Analogs in Dietary Supplements

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Abstract

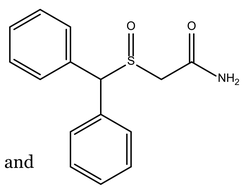
Drugs that boost concentration or wakefulness (nootropics, eugeroics) are prescribed for specific medical conditions. Nootropics have been used as recreational drugs and have also been found in dietary supplements. Modafinil (brand name Provigil®) is a Schedule IV drug used for the treatment of narcolepsy and sleep disorders in the U.S. Analogs of modafinil, including adrafinil, remain unapproved and/or unscheduled. The lack of scheduling has made these analogs a popular target for recreational use and inclusion in dietary supplements. However, the use of controlled substances without the care of a physician presents a public health risk. A preliminary untargeted analysis in our laboratory revealed the presence of adrafinil in several dietary supplements. A liquid chromatography high resolution mass spectrometry (LC-HRMS) method was developed to separate modafinil from four unscheduled modafinil analogs: adrafinil, bisfluoromodafinil (CRL-40,940), fladrafinil (CRL-40,941), and N-methyl-4,4-difluoromodafinil. The mass resolution of the Thermo Q-Exactive platform can differentiate unknowns of similar mass better than unit mass resolution instruments. The method discussed here can differentiate different modafinil analogs from one another, determining whether those drugs are scheduled drugs or unscheduled novel analogs. For this method, dietary supplement tablets and capsules containing no modafinil analogs were ground to a fine powder and used as blank matrix. The extraction consisted of shaking in acetonitrile for 30 minutes, followed by dilution of the supernatant. The extract was analyzed using a Thermo Q-Exactive Orbitrap, and identities were confirmed using retention time, exact mass of parent ions, and exact mass of fragment ions. This LC-HRMS method could be used alone or as a confirmatory method after suspected modafinil analogs are detected in our laboratory through HRMS untargeted screening of dietary supplements. The identification of modafinil and its analogs is important in this context so that consumers are not, knowingly or unknowingly, consuming these active pharmaceutical ingredients in dietary supplements.

Introduction

- Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide; brand name Provigil®) is a Schedule IV drug used for the treatment of narcolepsy and sleep disorders in the U.S.
- Analogs of modafinil, including adrafinil, remain unapproved or unscheduled.
- The lack of scheduling has made these analogs a popular target for recreational use and inclusion in dietary supplements.
- A preliminary untargeted analysis in our laboratory revealed the presence of adrafinil in several dietary supplements.

Why is this bad?

- Dietary supplements are regulated as a food under the FD & C Act.
- As such, they may only contain approved dietary ingredients.
- The presence of *any* active pharmaceutical ingredient (API) in a supplement constitutes adulteration.
- Modafinil (and presumably, its analogs) can have potentially serious side effects, including:
  - Headache/migraine
  - Nausea
  - Anxiety
  - Dizziness
  - Difficulty sleeping



- Untargeted screening useful for finding unknown unknowns in dietary supplements and can be useful in this context.

Materials and Methods

Extraction. 100 mg of supplement powder was weighed and 20 mL acetonitrile was added. The mixture was shaken for 30 minutes and then allowed to settle. 10 µL supernatant was diluted to 50 mL with a 90:10 mixture of water: acetonitrile containing 0.1% formic acid. The extract was subsequently separated on a Thermo Hypersil Gold C18 column (2.1 x 100 mm, 1.9 µm packing) on a Dionex UltiMate 3000 UPLC connected to a Thermo Q-Exactive Orbitrap.

LC-HRMS. The UPLC Conditions were as follows: Column Temperature: 30°C; Column Temperature Delta: 1.0°C; Autosampler Temperature: 5.0°C; Autosampler Temperature Delta: 1.0°C; Mobile Phase A: 0.1% formic acid in H2O; Mobile Phase B: 0.1% formic acid in acetonitrile; Flow rate: 300 µL/min (0.3 mL/min). The gradient program is shown in Table 1. Mass spectrometer conditions were as follows: Use lock masses: best; Chrom. peak width (FWHM) 15 s; Method duration 20.00 min. Additional mass spectrometer parameters are shown in Table 2.

Time (minutes)	%A	%B
0	82	18
2.0	82	18
15	70	30
17	5	95
19.5	5	95
20.0	82	18
20.1	82	18

Table 1. UPLC gradient parameters.

MS Parameter	Full MS-SIM	AIF	AIF (second instance)
Runtime	0.5 to 20 min	0.5 to 20 min	0.5 to 20 min
Polarity	positive	positive	positive
In-source CID	0.0 eV	0.0 eV	0.0 eV
Microscans	1	1	1
Resolution	70,000	70,000	70,000
AGC target	3e6	3e6	3e6
Maximum IT	100 ms	50 ms	200 ms
(N)CE/Stepped (N)CE	N/A	15, 35, 75	10, 20, 30
Scan range	100 to 1250 m/z	50 to 450 m/z	50 to 450 m/z
Spectrum data type	Profile	Profile	Profile

Table 2. Mass spectrometry parameters.

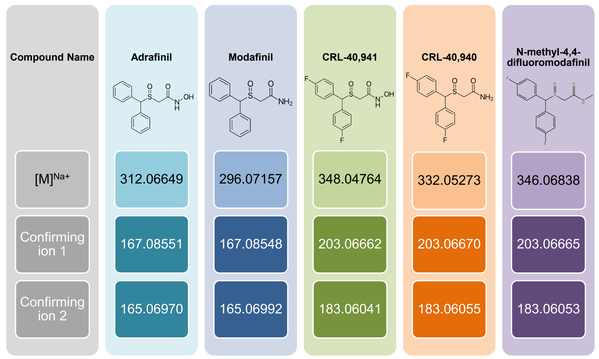


Figure 1. Analyte parent m/z and confirming ion m/z ratios.

Results and Discussion

Analyte	Spike	Tablet 1 (% RSD)	Tablet 2 (% RSD)	Capsule 1 (% RSD)	Capsule 2 (% RSD)
Adrafinil	Low	99.69	100.13	95.27	100.20
Adrafinil	Mid	96.99	99.25	92.63	98.55
Adrafinil	High	94.78	96.87	91.92	96.42
Modafinil	Low	107.50	115.56	105.23	110.18
Modafinil	Mid	103.76	110.47	98.28	105.93
Modafinil	High	92.50	120.58	88.39	96.16
CRL-40,941	Low	95.87	96.77	95.39	96.84
CRL-40,941	Mid	98.13	100.10	92.22	97.02
CRL-40,941	High	96.61	91.76	93.68	100.79
CRL-40,940	Low	98.58	107.15	98.15	100.87
CRL-40,940	Mid	95.98	106.22	95.13	95.92
CRL-40,940	High	90.36	111.37	91.79	97.74
N-methyl-4,4-difluoromodafinil	Low	105.02	112.06	105.78	108.76
N-methyl-4,4-difluoromodafinil	Mid	101.58	110.01	97.96	106.40
N-methyl-4,4-difluoromodafinil	High	91.64	112.37	91.12	95.47

Table 3. Method validation results: accuracy. Target: 80-120%.

Analyte	Spike	Tablet 1 (% RSD)	Tablet 2 (% RSD)	Capsule 1 (% RSD)	Capsule 2 (% RSD)
Adrafinil	Low	4.52	3.03	1.08	1.38
Adrafinil	Mid	3.46	3.61	1.14	1.22
Adrafinil	High	0.93	1.57	0.38	3.21
Modafinil	Low	2.16	1.50	2.95	1.94
Modafinil	Mid	1.16	1.45	1.10	1.70
Modafinil	High	0.86	1.45	0.62	2.16
CRL-40,941	Low	5.24	2.41	4.52	2.08
CRL-40,941	Mid	2.44	2.85	1.41	2.02
CRL-40,941	High	2.38	1.36	3.49	3.27
CRL-40,940	Low	5.31	5.38	6.95	2.82
CRL-40,940	Mid	4.22	4.81	3.73	5.76
CRL-40,940	High	5.31	4.63	3.26	2.82
N-methyl-4,4-difluoromodafinil	Low	2.27	1.65	3.38	1.69
N-methyl-4,4-difluoromodafinil	Mid	0.96	0.78	3.47	1.87
N-methyl-4,4-difluoromodafinil	High	0.92	1.11	1.39	3.17

Table 4. Method validation results: precision. Target:≤ 20%

Analyte	Tablet 1 (ng/mL)	Tablet 2 (ng/mL)	Capsule 1 (ng/mL)	Capsule 2 (ng/mL)
Adrafinil	0.905	0.880	0.627	0.884
Modafinil	0.628	0.518	0.348	0.324
CRL-40,941	2.417	2.730	1.775	1.281
CRL-40,940	1.099	1.309	1.601	1.133
N-methyl-4,4-difluoromodafinil	0.777	0.521	0.606	0.398

Table 5. Method validation results: method detection limit (MDL).

Analyte	Tablet 1 (ng/mL)	Tablet 2 (ng/mL)	Capsule 1 (ng/mL)	Capsule 2 (ng/mL)
Adrafinil	2.879	2.799	1.995	2.811
Modafinil	1.997	1.648	1.107	1.032
CRL-40,941	7.689	8.687	5.649	4.076
CRL-40,940	3.497	4.163	5.095	3.604
N-methyl-4,4-difluoromodafinil	2.473	1.658	1.927	1.265

Table 6. Method validation results: Limit of quantitation (LOQ).

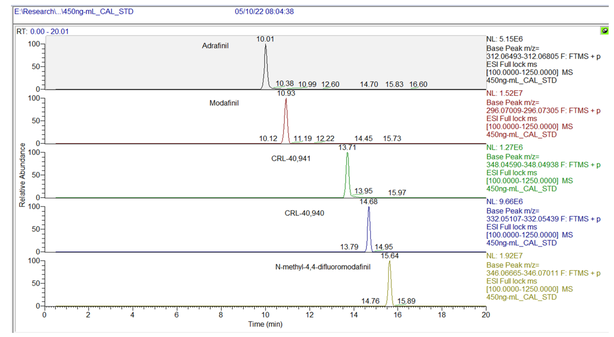


Figure 2. Each of the five analytes displays a unique retention time and parent ion m/z (sodium adduct), demonstrating specificity and selectivity.

Conclusion

QC Measure	Criteria	Reached?
Accuracy	80-120%	Yes
Precision	< 20% RSD	Yes
RT Precision	< 0.5 min RT shift	Yes
LOD	Evaluated	Yes
LOQ	Less than lowest calibrator	Yes
MDL	Evaluated	Yes
Linearity	≥ 0.995	Yes

Table 6. Summary of method validation results.

We have validated an analytical LC-HRMS method for the identification and quantitation of modafinil and four additional analogs in dietary supplements. LC-HRMS has been useful in identifying APIs such as adrafinil in supplements previously. This elegant method features both targeted and untargeted aspects, allowing us to detect and quantitate known analogs while also allowing for the identification of new analogs. This method is ready for use in regulatory work on dietary supplements.