# Rapid Screening of Dietary Supplements with Direct Analysis ID-CUBE Coupled to an Exactive Mass Spectrometer

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## Key Words
Exactive, DART, ID-CUBE, Screening, Dietary Supplements, Herbal

## Introduction

In Westernized cultures, dietary supplements have been gaining popularity in recent years. In China, traditional herbal supplements have been used for millennia. In both cases, the threat of adulteration and need for further characterization of ingredients have been driving forces behind the need for increased screening. This note presents a new approach to the rapid screening of a product marketed as an herbal weight loss aid and the fingerprint characterization of omega fatty acid dietary supplements derived from marine and flaxseed oils.

The Direct Analysis in Real Time (DART®) ionization source,[1] as well as other direct ambient ionization techniques such as Desorption Electrospray Ionization (DESI)[2] and the Atmospheric Solids Analysis Probe (ASAP®),[3] have gained momentum in recent years as more-robust and higher-throughput ionization techniques for mass spectrometry (MS) in routine markets. None of these direct ionization techniques incorporate online sample separation. As a result, a vast number of ions can be generated from what are often complex samples. Thus there is great benefit to coupling DART and other direct ambient ionization sources with high-resolution mass spectrometers that can distinguish ions of interest from those of the matrix and background interferences. Thermo Scientific Orbitrap mass analyzer technology with >100,000 mass resolution (full width at half maximum – FWHM – at *m/z* 200) and <5 ppm mass accuracy (RMS, with external mass calibration) easily achieves the levels needed for this task.

The ID-CUBE® is a next-generation DART ion source that instantaneously desorbs and ionizes analytes off standardized sample cards called OpenSpot® cards. By resistively heating the stainless steel mesh sampling surface of the OpenSpot card, the source desorbs the analytes directly into the reactive helium gas stream where they are ionized via the DART atmospheric pressure chemical ionization (APCI) processes.[1, 4-7]

The ID-CUBE can directly sample both liquids and powders. It fully integrates with the mass spectrometer without the need for stand-alone software control. The overall performance and robustness of the ID-CUBE/Orbitrap™ mass analyzer combination is demonstrated here through rapid screening of dietary supplements and an herbal weight loss aid.

## Experimental

### ID-CUBE/Exactive MS

The ID-CUBE ionization source (Figures 1-3), was coupled to a Thermo Scientific Exactive benchtop Orbitrap mass spectrometer using a Vapur® gas ion separator interface[8]. The ID-CUBE discharge voltage was controlled through Thermo Scientific Xcalibur software by setting the ion source spray voltage to 1.0 kV and the sheath, auxiliary and sweep gases to zero for all ID-CUBE analyses. The Exactive™ MS was operated in positive and negative ion modes with the following scan and inlet parameters:

| | |
|---|---|
| Number of microscans: | 1 |
| Maximum inject time: | 250 ms |
| Automatic gain control (AGC) target: | 1,000,000 |
| Capillary temperature: | 200 °C |
| Capillary voltage: | +25/-50 V |
| Tube lens voltage: | +/-120 V |
| Skimmer voltage: | +/-25 V |

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Figure 1. ID-CUBE direct analysis in real time (DART) ionization source coupled with an Exactive MS

External mass calibration was performed at the beginning of the week in which the analyses took place using positive and negative ion calibration solutions prepared according to the protocol in the Exactive MS operating manual. The calibration was performed by direct infusion using the heated electrospray (HESI) Thermo Scientific Ion Max source. The resolving power setting for the Exactive MS was set at 50,000 FWHM (at *m/z* 200) resulting in 2 scans per second.

The helium gas flow for the ID-CUBE was set at 2 SCFH (standard cubic feet per hour), corresponding to 1.14 L/min. The ID-CUBE source was operated with OpenSpot sample cards that consisted of a small piece of metal mesh with a narrowed sample spotting area. The OpenSpot cards were inserted into the slot on the top of the ID-CUBE source (Figure 2) and the heating setting was selected on the ID-CUBE switch box. All analytes were thermally profiled using all three desorption settings of Low (180 °C), Medium (360 °C) and High (580 °C) before running replicate samples. Data acquisition was set up through an Xcalibur™ software sequence. The ID-CUBE source triggered the mass spectrometer data acquisition via a contact closure.

Figure 2. OpenSpot sample card and introduction to the ID-CUBE ion source

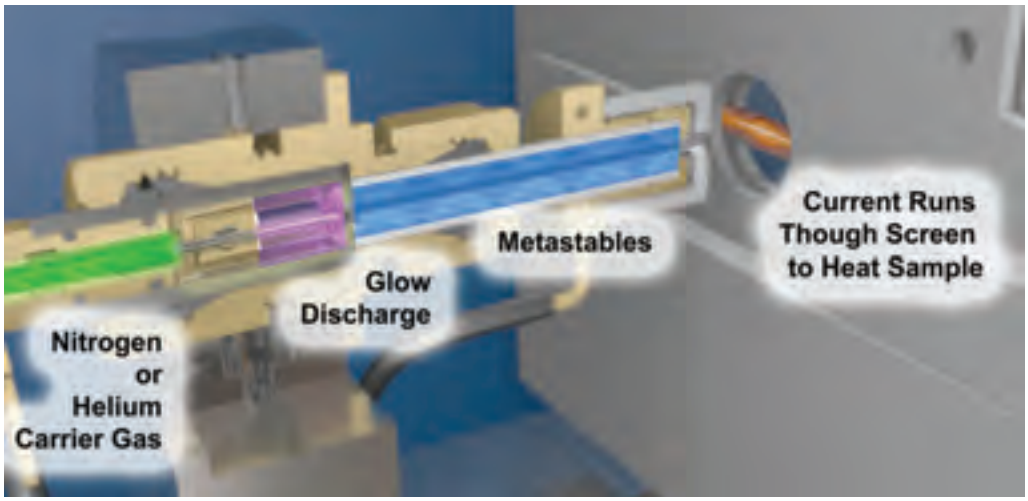


Figure 3. Schematic diagram of the ID-CUBE ambient ionization source

### Analysis of Counterfeit Herbal Weight Loss Aid

Pai You Guo is a commercial Chinese herbal weight loss aid. In 2009, Pai You Guo was withdrawn from the market by its manufacturer[9] after FDA investigation determined that it contained sibutramine, an FDA-regulated appetite suppressant used for weight loss, as well as other unapproved compounds. Sibutramine itself was subsequently withdrawn from the market in the European Union, United States, and other countries. In 2010 and 2011, articles in the Boston Globe newspaper[10,11] indicated that Pai You Guo was still available through stores and over the Internet and was still being used by consumers.

For this research, Pai You Guo was purchased online in August 2011 from a vendor based in the United States. The packaging for the Pai You Guo did not state anywhere that sibutramine could be among the ingredients of the capsules.

The Pai You Guo capsules contained a very fine powder and were twisted open to directly sample the powder. Using a closed end melting point glass capillary tube, a very small amount of the powder was pressed onto the end of the glass tip and this material was scraped across the sampling area of an OpenSpot card as shown in Figure 4. The powder was thermally profiled and a heating setting of Medium was selected. For comparison, a small amount of the capsule powder was also dissolved in acetonitrile (ACN) and 5-µL aliquots were sampled from the OpenSpot cards.

Figure 4. OpenSpot cards used for liquid and solid sample application

### Analysis of Omega Fatty Acid Dietary Supplements

For this research, several omega fatty acid dietary supplements were purchased locally. The omega-3, 6 and 9 fatty acids were compared between two fish oil dietary supplements (Nature Made and Nature's Bounty), MegaRed krill oil, and Nature Made triple omega dietary supplement, which contained a mixture of fish oil, safflower oil and flaxseed oil.

A similar comparison was conducted for the fingerprint fatty acid components of several flaxseed oils including Nature Made triple omega, Nature Made flax oil and Target Origin flax oil.

Preparation of these oils for ID-CUBE analysis consisted of performing a 2% dilution in toluene and spotting 5 µL of the diluted solutions directly onto an OpenSpot sample card.

## Results and Discussion

### Herbal Weight Loss Aid

Of the 30 capsules in the Pai You Guo package, all five capsules sampled tested positive for the presence of sibutramine. Figure 5 shows the spectra obtained from the ID-CUBE direct analysis of the capsule powder and analysis of a saturated solution of the powder. The top spectrum in Figure 5 displays the simulated $[M+H]^+$ spectrum for the elemental composition $C_{17}H_{26}NCl$ for sibutramine. The signal from the capsule powder sampled directly for the $[M+H]^+$ ion at *m/z* 280.18204, with mass accuracy of -2.2 ppm dominated the spectrum with all other peak intensities less than 15%. The capsule contents sampled as a saturated solution generated more intense signal, approximately an order of magnitude greater than the direct powder analysis because a greater amount of the capsule material was sampled. The mass accuracy reported for the analysis of the saturated solution was -2.7 ppm.

For further confirmation that sibutramine was detected in the Pai You Guo capsules, the saturated solution was subjected to an all-ion fragmentation (AIF) experiment. The higher-energy collisional dissociation (HCD) cell was used at 20 eV to fragment all ions without precursor ion isolation. The MS1 full-scan spectrum was dominated by the $[M+H]^+$ ions for sibutramine and, as a result of applying the AIF, a relatively simple fragmentation spectrum was recorded as shown in Figure 6. The major fragment ion $C_7H_6Cl$ at *m/z* 125.01518 with mass accuracy of -0.6 ppm, as well as a minor fragment $C_8H_8Cl$ at *m/z* 139.03071 with mass accuracy of -1.3 ppm, maintained the chlorine isotope pattern and resulted from losses of $C_{10}H_{21}N$ and $C_9H_{19}N$ respectively.

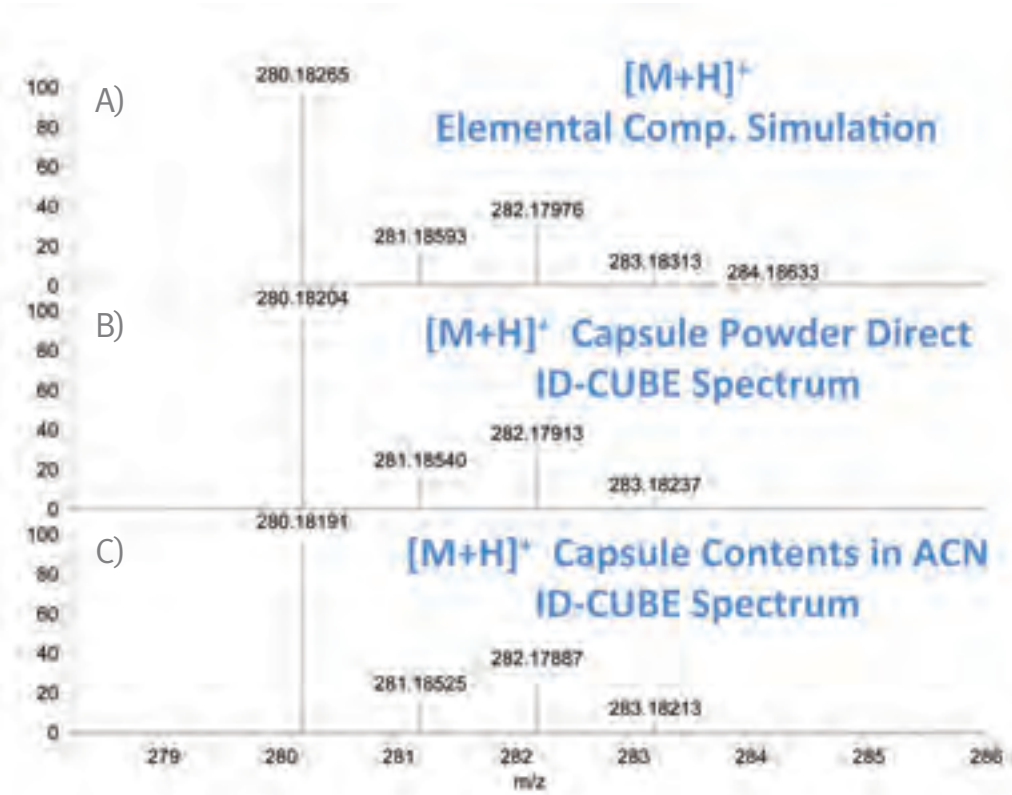


Figure 5. Mass spectra for the analysis of Pai You Guo herbal weight loss aid.

A Elemental composition simulation for sibutramine $[M+H]^+$ at *m/z* 280.18265.

B Direct analysis of the Pai You Guo capsule powder, delta ppm of -2.2 ppm.

C Analysis of 5 µL of a saturated solution in acetonitrile (ACN), delta ppm of -2.7 ppm.

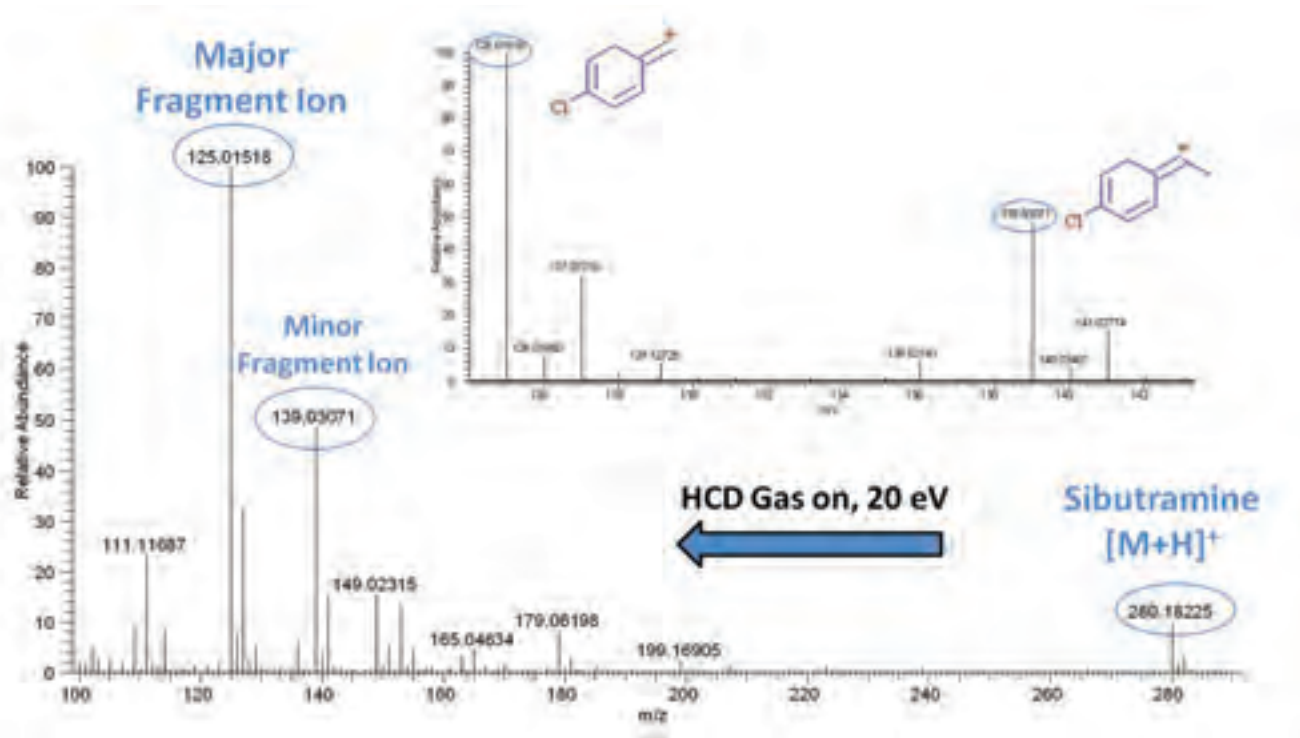


Figure 6. HCD experiment on Pai You Guo capsule content. Sibutramine $[M+H]^+$ ions ($C_{17}H_{27}NCl$) was fragmented with HCD gas on and 20 eV producing a major fragment $C_7H_6Cl$ at *m/z* 125.01518 and a minor fragment $C_8H_8Cl$ at *m/z* 139.03071.

## Omega Fatty Acid Dietary Supplements

Another area of great interest with regard to dietary supplements is the use of marine-and plant-based oils as a source of essential omega fatty acids. Competing products in marine oil dietary supplements, namely fish oils, are being challenged by the manufacturers of krill-based oils. Krill oil dietary supplement manufacturers position their products as being superior to fish oil products for the relative amounts omega-3 essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These experiments profiled two fish oil dietary supplements, one plant and fish omega-3 mixture, and one krill oil omega-3 dietary supplement. The oils were not sampled directly from the supplement capsules because the concentrations are extremely high. A 2% dilution in toluene permitted a thin coating of material to be evenly applied onto the mesh of the OpenSpot sample card. The ID-CUBE heating setting was optimized in less than one minute and all of the samples were analyzed on a Medium heating setting. Sample analysis time per OpenSpot card was 10 seconds.

Figure 7 displays the results of the ID-CUBE analysis of diluted oil supplements where all four supplements contained varying levels of DHA and oleic acid. The average area (n=5) for EPA was normalized to 1, yielding relative ratios for DHA and oleic acid. The two fish oils contained the most DHA per capsule and the Nature's Made Triple Omega supplement, which was a mixture of plant and fish oil, contained nearly the same level of DHA as the krill oil per capsule. Reproducibility RSD values for replicates of five for EPA was 9% +/- 1%, normalizing the signal to oleic acid signal. For the less intense DHA signal, reproducibility for replicates of five was 19% +/- 3%.

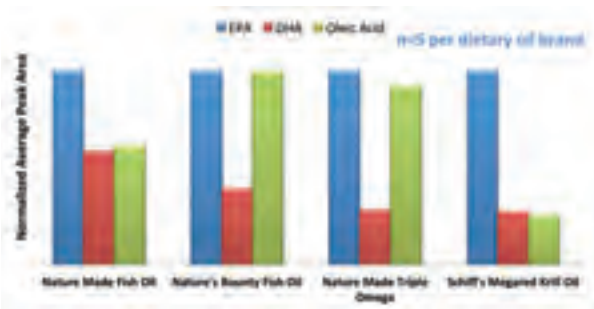


Figure 7. Analysis of marine oil samples monitoring omega-3 essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as omega-9 oleic acid. All of the oils were sampled as a 2% solution in toluene and EPA area was normalized to 1, n = 5.

Figure 8 focuses on plant-based essential omega fatty acids α-linolenic and linoleic acids in flaxseed oils and the triple omega oil mixture. Flaxseed oil is one of the richest sources of α-linolenic acid. The name brand flaxseed oil supplement by Nature Made analyzed against the store brand Target Origin flaxseed oil yielded nearly identical ratios of α-linolenic and linoleic acids. The triple omega oil mixture contained roughly half the amount of α-linolenic as the pure flaxseed oil supplements and the linoleic acid level was nearly the same per capsule. Figure 9 shows an example mass spectrum of Omega-3, 6 and 9 fatty acids from Nature Made Triple Omega dietary supplement containing flaxseed, safflower and fish oil product.

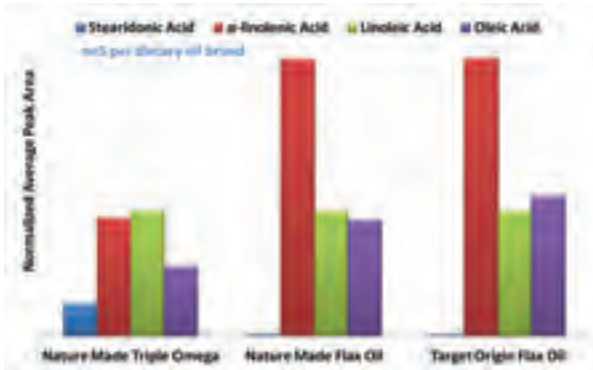


Figure 8. Omega-3, 6 and 9 fatty acid analysis of three flaxseed oil products. All of the oils were sampled as a 2% solution in toluene, n = 5.

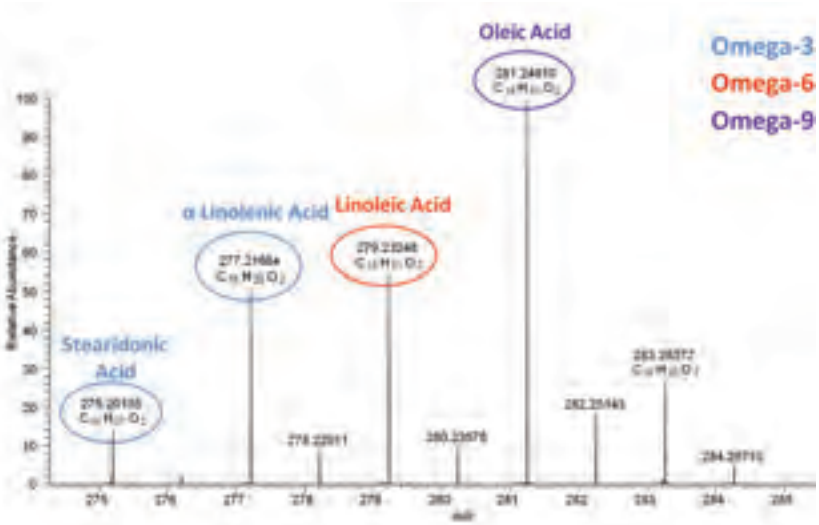


Figure 9. Mass spectrum of Omega-3, 6 and 9 fatty acids from Nature Made Triple Omega dietary supplement containing flaxseed, safflower and fish oil product

## Conclusions

The combination of the ID-CUBE coupled to the Exactive high-resolution MS proved to be a rapid and robust tool for screening and fingerprint characterization. The Orbitrap platform provided extremely fast accurate-mass measurements of <3 ppm with external mass calibration for all of the analyses, and switching between the ESI source and the ID-CUBE source was very smooth, taking less than 3 minutes total. Optimization of the heating setting was achieved in less than one minute for rapid heating of the sample. Using the standardized OpenSpot sample cards, a single sample analysis could be completed in 10 seconds per card. Both liquids and powders were easily analyzed using the ID-CUBE source as shown in the experiments detecting sibutramine in Pai You Guo herbal weight loss aid. Dietary supplements could be quickly fingerprinted and characterized for quality control testing.

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