The One-Pass-Flash Method to Test Supplements for Anabolic Androgenic Steroid Adulterations

James Neal-Kababick, Flora Research Laboratories, Grants Pass, Oregon, USA

Introduction

Flora Research Laboratories developed a rapid and simple “One Pass Flash” method for Anabolic Androgenic Steroids in dietary supplements. Tribulus terrestris is a highly complex, natural product that is standardized on sterol content. Typically, commercial material contains 45% sterols. On more than one occasion, the use of this material has caused false positive hits for AA steroids by vibrational spectroscopy (FTIR) and LC-MS/MS triple quad analysis. Standard SPE cleanup methods utilized in anti-doping labs are not suitable for this matrix since the process also extracts the sterol fraction, creating a highly complex matrix for analysis. Following on the tremendous success of our work with One Pass Flash Chromatography of PDE-5 Inhibitors in complex botanical matrices, we developed a simple and rapid approach suitable for Anabolic Androgenic Steroids.

Experimental

Standard Test Mix

A mixture of 5 anabolic steroids was prepared in chloroform and loaded onto silica gel (Davisil® silica gel). This was fractionated utilizing a gradient of dichloromethane-methanol over 15 minutes. The method showed that all of the steroids eluted in a single fraction on a 4 gram cartridge from 1.9 – 2.8 minutes. Other fractions tested positive for steroids as well but were very minor and determined to be impurities from the standard mixture. The standard mixture fraction was evaporated to near dryness, filtered, and analyzed by GC-MS. The GC-MS data indicated that all five anabolic steroids were recovered in that fraction. (See GC-MS trace).

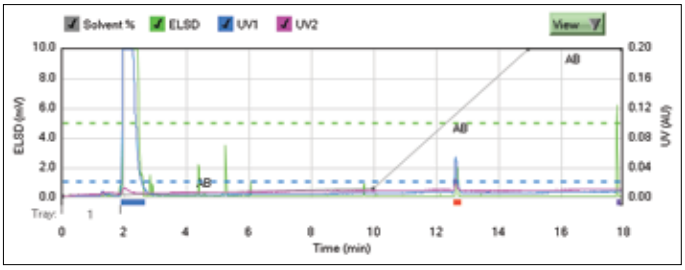

Figure 1: Flash Chromatogram of Steroid Mixture Showing Major AAS Fraction

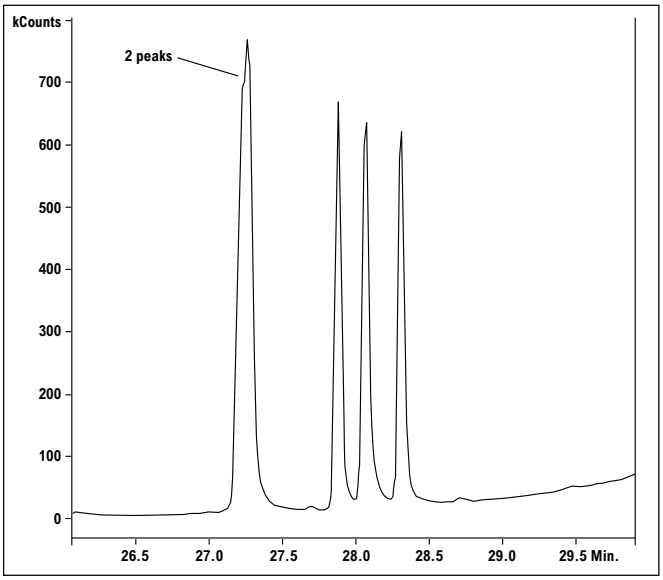

Figure 2: GC-MS Analysis Showing All 5 AAS Peaks Recovered from Fraction Using “One Pass Flash” Technique

Test Sample Preparation

Approximately 500 mg of tribulus extract sample was extracted into 20mL of $CHCl_3$ in a glass centrifuge tube, first by shaking on a wrist action shaker for 15 minutes then by sonication for 15 minutes. The sample was centrifuged and submicron filtered into an amber vial for flash. About 2.5 grams of Davisil® silica gel was weighed into a 50mL round bottom flask; the filtrate from the extract above was added to this flask and mixed. The flask was evaporated (Rotovap) to dryness under vacuum and the free flowing silica powder containing the sample was packed into a sample loading cartridge. The sample was analyzed as above.

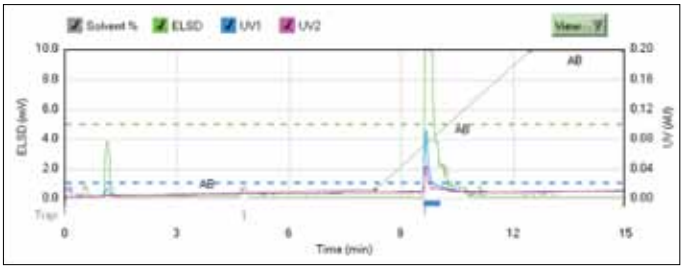

Figure 3: Test Sample Showing No Anabolic Steroids. Major Peak is Fatty Acid Matrix

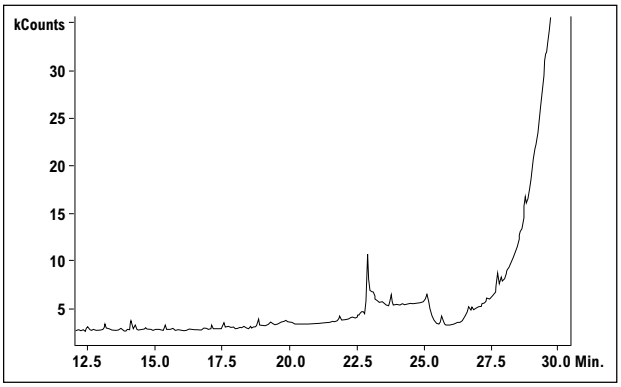

Figure 4: Chromatogram of Fraction Showing Fatty Acids and Fatty Acid Amides (No AAS)

Results

No fraction or peak was observed in the anabolic steroid window although a small peak pair was observed eluting earlier and later, but these peaks were too low to trigger the fraction collection. However, a late eluting peak around 9.1 – 9.6 minutes was collected, eluting off at approximately 40% methanol (strong solvent). Based on this experiment, the sample is negative for anabolic androgenic steroids and no further work up or analysis is required.

The resulting chromatogram of the late eluting fraction showed that the fraction was composed mostly of fatty acids, but it was interesting to observe that a fatty acid amide was found in the product as well. An examination of the mass spectra of the peaks showed that no anabolic androgenic steroid pattern was found.

Discussion

Once again, Flora Research Laboratories has utilized the Grace® Reveleris® X2 Flash Chromatography System with our “One Pass Flash” Technique to obtain highly concentrated and purified fractions for analysis by mass spectroscopy. In this particular application, the data in the chromatogram obtained by the system was used to determine the absence of anabolic androgenic steroids (since no peak was detected/collected) in the time window shown to contain these compounds.

The technique allows for a clean fraction to be injected (when collected) that greatly reduces instrument down time at the mass spectrometer from dirty matrix-heavy samples. The quick and easy process can be accelerated by stopping the run after 5 minutes since the fractionation column is an inexpensive consumable and is not reused.

Conclusion

Over the last two years, Flora Research Laboratories has tested a number of products that tested false positive using traditional sample preparation methods and analytical techniques in other laboratories. These techniques (other laboratory methods using traditional sample prep) were not originally developed to handle the highly complex matrices found in dietary supplements. “One Pass Flash” sample preparation, as shown here, can even circumvent the need to go to higher level analysis when no peak collection is triggered in the window(s) for the banned substances.

As laboratories involved in the testing of banned substances continue to work with increasingly complex botanical and dietary supplement matrices, the “One Pass Flash” method of sample preparation will become essential to avoid false positives and excessive down time on LC-MS and GC-MS systems.

Americas
Deerfield, Illinois
Tel: +1 847.948.8600
discoverysciences@grace.com

Europe
Lokeren, Belgium
Tel: +32 (0)9.340.65.65
discoverysciences.EU@grace.com

India
Pune
Tel: +91 20.6644.9900
pune@grace.com

China
Shanghai
Tel: +86 21.5467.4678
dsbiz.asia@grace.com

Australia
Rowville
Tel: +61 3.9237.6100
discoverysciences.AU@grace.com

www.discoverysciences.com

04/13 M393a