

Nordic Naturals/Nutrasource Diagnostics, Inc.
True Anisidine Value Test: (TAV) Test Backgrounder

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BACKGROUND

As the leading supplier of omega-3 products, Nordic Naturals demonstrates its commitment to the industry by devoting time and resources to standards that continuously improve the quality of fish oil in the natural products marketplace. In partnership with Nutrasource Diagnostics, Inc. (NDI), a leading research organization, Nordic Naturals has guided the development of a new testing methodology, the True Anisidine Value (TAV) Test, which properly distinguishes lipid oxidation from other factors that interfere with this measurement in finished omega-3 formulas. This groundbreaking method is able to analyze p-Anisidine levels in marine- and plant-based oils by overcoming each of the disadvantages that had confounded conventional testing methods, which often led to false positives for rancidity in flavored formulas. The new method, based on HPLC analysis, provides methodological sensitivity, robustness, and accuracy to quantify the oxidative status of marine omega oil products. This technology is important not just for Nordic Naturals, but for the entire industry as well.

FRESHNESS AND RANCIDITY OF FISH OIL

All oils are susceptible to rancidity, which occurs when there is decomposition of the oil. Over a long enough period of time, all oils undergo this process when exposed to damaging conditions. Marine oils are no exception, and, as a result, are highly susceptible to degradation if handled improperly during either manufacturing or storage. Rancidity in fish oil can result in poor taste and smell, and may potentially damage the beneficial fatty acids present in the oil. Exposure to oxygen is the main driver of rancidity in fish oil products, resulting in compromised taste.

Most people can detect damaged fish oil simply by its smell, but there are also chemical tests to measure exact freshness levels. The most commonly used chemical tests are Peroxide Value and p-Anisidine Value. Peroxide is the measurement of peroxide oxygen present. It is a single picture of the oil's current state of rancidity. Anisidine value (AV) is a measurement of secondary oxidative products, specifically aldehydes, which are the drivers of poor taste and smell. AV measurements provide a snapshot of the oil's history, compared to the shorter timeframe indicated by PV. Unfortunately, AV testing has remained limited in application, as the addition of flavors, colors, preservatives, or vitamins to the oil itself can alter the measurement of aldehydes, giving a false indication of freshness. This limitation is the first numbered note in the AOCS method p-Anisidine method 18-90.

Freshness is further measured by AV and PV, which are combined to reflect a TOTOX, or total oxidation value. TOTOX results are skewed by the addition of a number of components, including lemon flavoring; however, it does not mean that the oil is oxidized. Nordic Naturals uses a patented manufacturing process to produce great-tasting flavored oils. The natural citrus flavors used in some of our oils naturally contain an aldehyde. Aldehydes have a type of chemical bond that also exists in fish oil. Aldehydes in fish oil can increase when a fish oil product is oxidized and goes rancid. However, aldehydes found in flavoring agents such as the citrus flavors used by Nordic Naturals help maintain a specific flavor profile and scent. Thus, the presence of aldehydes due to flavoring may cause an artificially high anisidine value that is not related to oxidation and does not represent the freshness of the oil.

Oxidation levels traditionally have been measured by analyzing samples for elevated levels of p-Anisidine using AOCS, AOAC, and/or USP standardized methods. However, until now there has been no industry standard for testing flavored fish oil products. All Nordic Naturals non-flavored oils are tested for AV after encapsulation, and all flavored oils are tested for AV based on our refined oils/pre-flavored oils, as only flavored products need to be tested for AV prior to flavoring and encapsulation. All other tests, including peroxide value, purity, etc., are completed after encapsulation.

THE NEW TESTING METHODOLOGY

The limited utility of the existing AV test, and industry confusion over when to use it, drove Nordic Naturals and NDI to create the new TAV test. This testing methodology is the first chemical freshness test to measure rancidity of secondary oxidative products in *flavored* oils at the final encapsulation point. Nordic Naturals has been flavoring oils for decades, and is committed to delivering the best-tasting and freshest omega-3 products available. The TAV test advances the industry by introducing an accurate measurement of freshness of flavored oils, providing greater overall transparency and increased confidence on the part of customers.

The TAV test is a method using HPLC analysis to measure the reduction of a p-anisidine standard after a reaction with a prepared sample. The peak is measured, and a percentage of anisidine remaining is established; the higher the percentage of remaining anisidine, the fresher the oil. The use of an HPLC and the simplicity of the method make this an accessible test for omega-3 manufacturers to implement and for labs to conduct.

The level of rancidity is the final step in determining the freshness of the oil by the TAV test. It has always been difficult to establish the point at which an oil is, in fact, rancid because this involves quantifying what amounts to the subjectivity of taste. For the TAV test, NDI and Nordic Naturals have established a standard below which an oil is considered rancid. This standard was validated by NDI, which conducts hundreds of fish oil freshness tests each year through their internationally recognized IFOS program. The addition of the TAV test to the IFOS program for flavored fish oils will assist in developing the TAV test as an industry standard.

The Global Organization for EPA and DHA Omega-3 (GOED) and the Council for Responsible Nutrition (CRN) have established industry monographs for fish oil, and representatives of these groups are excited about the inclusion of the new TAV test as a way to move the omega-3 industry forward with better testing for freshness. Inclusion by the AOCS (American Oil Chemists Society) or a pharmacopeia will be the next step in method acceptance.

Nordic Naturals and NDI are very excited to advance the industry in this new direction, and to help ensure that the products we deliver to consumers are the freshest possible. NDI president and CEO William Rowe states, “The marine oil industry has long been technically challenged by flavored fish oils in determining if par-anisidine results under the AOCS method are false positives or truly rancid fish oils. The True Anisidine Value test as co-developed by Nordic Naturals and Nutrasource Diagnostics, Inc. will revolutionize the way in which flavored oils are tested and substantiated for stability. It provides a direct measurement of stability free of the interferences traditionally found in the AOCS method. In addition to the method development, validation, and reference point established, Nutrasource Diagnostics, Inc. is and will be working diligently in having this method adopted by industry, chemistry organizations, and related trade groups so that this test becomes the new gold standard for testing of flavored marine oil products.”

THREE-PHASE COMMERCIALIZATION PLAN

Phase 1

- a. NDI will incorporate the TAV test into the IFOS program for fish oils containing flavorants, antioxidants considered as possible flavorants, and combination products, which contain ingredients that may interfere with traditional AOCS testing.
- b. NDI will offer the test to the global market. The current price is \$300 USD; however, steps are in place to reduce this price as volume grows.
- c. NDI will work with industry groups to raise awareness of the TAV test, and the solutions it provides, and to encourage its inclusion in certain key monographs as an option for the classes of products described in 1.a (Main monograph target is the GOED monograph; main awareness-raising trade associations are CRN and NPA).

Phase 2

- a. NDI will work with trade association members, who may be raw material producers, formulators, encapsulators, flavorant suppliers, or branders, to set up the TAV test at their facilities.
- b. NDI will conduct an ongoing proficiency-testing program to confirm and verify that the method is being properly followed at in-house laboratories that have received the TAV test.

Phase 3

- a. NDI will endeavour to establish the TAV test as a numbered method with chemistry associations such as AOCS, AOAC, and USP.