Molecular Enzymology and Drug Targets

Extended Abstract
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Pharma – 2012 :Rapid fatty acid and microbial identification using the MIDI sherlock® microbial identification system -Michael Alexander - MIDI Inc.

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MIDI, Inc. is a privately held, veteran-owned biotechnology company based in Newark, Delaware, USA. The company has been developing rapid microbial identification products and solutions for environmental and clinical microbiology laboratories since1991. The company was founded by Dr. Myron Sasser, former Professor of Plant Pathology at The University of Delaware, USA. Microbiology laboratories worldwide have relied on the MIDI Sherlock® Microbial Identification System (MIS) for rapid microbial identification and phospholipids fatty acid analysis (PLFA). The Sherlock MIS technology uses gas chromatographic analysis of fatty acid methyl esters (GC-FAME). The Sherlock MIS Software controls a MIDI-configured Agilent Technologies GC, names the individual fatty acids in the sample and identifies the sample by comparing the GC-FAME profile to stored libraries of well-characterized strains. Post sample analysis can be used for strain tracking and microbial community profiling.

The Sherlock MIS can be used in conjunction with several sample preparations depending on customer need, or a customer can develop their own protocol and libraries. The 90 minute Traditional method is the most well-established and is in use by most customers. Instant FAME™ was released in 2007 and is a 3 minute sample preparation method geared to environmental laboratories. Q-FAME™ was released in 2010 and is a 15 minute sample preparation method geared to clinical laboratories. Looking toward the future, MIDI is focusing efforts on non-microbial ID solutions, such as PLFA. A new PLFA method, peak naming table and calibration standards were released in 2012.

In chemistry, particularly in biochemistry, an acid may be a carboxylic acid with an extended aliphatic chain, which is either saturated or unsaturated. Most present fatty acids have an unbranched chain of a good number of carbon atoms, from 4 to twenty-eight. Fatty acids are usually not found in organisms, but instead as three main classes of esters: triglycerides, phospholipids, and cholesteryl esters. In any of those forms, fatty acids are both important dietary sources of fuel for animals and that they are important structural components for cells. Most present fatty acids have an unbranched chain of carbon atoms, with a carboxyl (–COOH) at one end, and a methyl (–CH3) at the opposite end. The carbon next to the carboxyl is labeled as carbon □ (alpha), using the primary letter of the Greek alphabet. Subsequent is labeled as □ (beta), then forth. Although fatty acids are often of diverse lengths, the last position is usually labelled as □ (omega), which is that the last letter within the Greek alphabet. The position of the carbon atoms within the backbone of a carboxylic acid are often also indicated by numbering them, either from the □COOH end or from the □CH3 end of the carbon chain. If the position is counted from the □COOH end, then the C-x notation is employed, with x=1, 2, 3, etc. (blue numerals within the diagram on the proper, where C-1 is that the □COOH carbon). If the position is counted from the □CH3 end, then it's represented by the □-x notation, or equivalently, by the n-x notation (numerals in red, where □-1 or n-1 refers to the methyl carbon). The positions of the double bonds during a carboxylic acid chain can, therefore, be indicated in two ways, using the C-x or the □-x notation. Thus, in an 18 carbon carboxylic acid, a covalent bond between C-12 (and □-7) and C-13 (or □-6) is reported either as Δ12 if counted from the □COOH end, or as □-6 (or omega-6) if counting from the □CH3 end. In both cases, only the "beginning" of the covalent bond is indicated.

A variety of such OTC medical products is now widely available. However, topically applied vegetable oil wasn't found to be inferior during a "randomised triple-blind controlled non-inferiority" trial conducted in Spain during 2015. Commercial products are likely to be less messy to handle and more washable than either vegetable oil or petrolatum, both of which, if applied topically may stain clothing and bedding. Hydrogenation of unsaturated fatty acids is widely practiced. Typical conditions involve 2.0–3.0 MPa of H2 pressure, 150 °C, and nickel supported on silica as a catalyst. This treatment affords saturated fatty acids. The extent of hydrogenation is indicated by the iodine number. Hydrogenated fatty acids are less prone toward rancidification. Since the saturated fatty acids are higher melting than the unsaturated precursors, the method is named hardening. Related technology is employed to convert vegetable oils into margarine. The hydrogenation of triglycerides (vs fatty acids) is advantageous because the carboxylic acids degrade the nickel catalysts, affording nickel soaps. During partial hydrogenation, unsaturated fatty acids are often isomerized from cis to Tran's configuration. Short- and medium-chain fatty acids are absorbed directly into the blood via intestine capillaries and travel through the hepatic portal vein even as other absorbed nutrients do. However, long-chain fatty acids aren't directly released into the intestinal capillaries. Instead they're absorbed into the fatty walls of the intestine villi and reassemble again into triglycerides. The triglycerides are coated with cholesterol and protein (protein coat) into a compound called a chylomicron. From within the cell, the chylomicron is released into a lymphatic capillary called a lacteal, which merges into larger lymphatic vessels. It's transported via the systema lymphaticum and therefore the lymph vessel up to a location near the guts (where the arteries and veins are larger). The lymph vessel empties the chylomicrons into the bloodstream via the left vena subclavia. At now the chylomicrons can transport the triglycerides to tissues where they're stored or metabolized for energy.

This work is partly presented at 2nd International Conference and Exhibition on Pharmaceutical Regulatory Affairs, November 23-24, 2012 held at Hyderabad International Convention Centre, India

Biography

Mr. Michael B. Alexander is the Director of MIDI's Training Operations, MIDI's Senior Support Engineer and Asia- Pacific Senior Speaker. He has given over 25 international talks (mostly in Asia) and conducted many MIDI Training Courses throughout the globe in the last 10 years. Mr. Alexander being MIDI Team Leader for our HPLC and PLFA Development Programs. Was involved with creating the new PLFA method, and was involved in the development of a new PLFA extraction protocol created with Dr. Jeffrey Buyer at the USDA-ARS (Beltsville, MD USA). This is widely accepted in the Agriculture and Plant Pathology communities, Agriculture Research Service (USDA-ARS) (United States Department of Agriculture-United States Department of Agriculture), where the MIDI Sherlock® System is used primarily for plant and soil analysis. Mr. Alexander has vast experience with gas chromatographic (GC) and high performance liquid chromatographic (HPLC) analysis of fatty acids for microbial identification and manufactures all consumables for these systems

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