Dynamic Adsorbents

Role of Chromatography in the Purification of Nutraceuticals

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New FDA Guidelines Mandate Highlight Importance of this Essential Analytical Technique

The enforcement role of the Food and Drug Administration will strengthen during the years of the Obama administration, assuring the public of a safer food and drug supply. Excitement is generating in the world of science that after the legacy of the Bush years the pharmaceutical and life science industries may once again be looked upon as economic growth engines spurring the creation of thousands of well paying jobs.

Dietary supplements may improve a sense of well being as well as actual health. These products are widely used by Americans, with an estimated 55% of adults taking some form of dietary supplement in any given month. In a 2001 poll performed by Harris Interactive (Rochester, NY) 72% of those surveyed take supplements to feel better, 67% to prevent illness, 50 % to live longer, 37% to build muscle and strength, 12% for weight management and 33% on the advice of a physician. Offering well characterized and quality formulated dietary supplements is a growth industry, with nutraceuticals and dietary supplements now being a $ 182 billion global market (40% of this market within the US alone). Products extracted and purified from botanical compounds account for 25% of this market share.

Among the dietary supplementary products growing in popularity are nutraceuticals. A nutraceutical is a made up word combining the words nutrition and pharmaceuticals, creating the concept that extracts from foods can be used as drugs. The term was created in 1989 by Dr. Stephen DeFelice of the Foundation for Innovation in Medicine. Nutraceuticals are often referred to as phytochemicals or functional foods. They are natural bioactive chemical compounds that have health promoting, disease preventing or medicinal properties. The American Nutraceutical Association notes that the term has been modified by Health Canada to mean "a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease." Significantly, 53% of those polled by Harris Interactive believed that nutraceuticals offer benefits not matched by conventional drugs and 56% said they offered benefits comparable with drugs but with fewer side effects.

The FDA has enacted tighter regulatory control for the manufacture of nutraceutical compounds – precisely because these compounds have the ability to provide benefit. The Dietary Supplement Health and Education Act (DSHEA) became law in 1994 amending the Federal Food, Drug and Cosmetic Act of 1938. This law places under FDA regulatory control the labeling and manufacture of dietary supplements. The goal of this legislation is to both increase consumer access to dietary supplements as well as provide the government with additional authority to regulate the industry. The legal enactment for full compliance with current Good Manufacturing Practices (cGMPs) (identical to those required of traditional ethical pharmaceutical manufacturers) went into effect for larger nutraceutical firms on June 25, 2008 and will be enforced for all nutraceutical manufacturing firms commencing June, 2010.

Companies with 500 or more full time equivalent employees are now subject to FDA inspection for compliance with this rule. Companies with 20 to 499 full time equivalent employees have until June 25, 2009 while those with less than 20 employees have until June, 2010 to be in full compliance. DSHEA expressly grants FDA the authority to promulgate current Good Manufacturing Practice (cGMP) regulations for dietary supplements. Specifications must be set and met for limits on contaminants, such as heavy metals, solvent residues and microorganisms in finished products. DSHEA codified the presumption that dietary supplements are generally perceived to be safe and only need regulation when clear evidence exists that they present a substantial or unreasonable risk of harm. DSHEA defines 5 categories of substances that qualify as dietary ingredients: vitamins, minerals, herbs, amino acids and dietary substances used to increase the total daily intake along with concentrates, metabolites, constituents, extracts or combinations of these ingredients. A dietary supplement as legally defined by DSHEA is "any product taken by mouth that contains a so called "dietary ingredient" and its label clearly states that it is a dietary supplement". DSHEA expanded the meaning of the term "dietary supplement" beyond essential nutrients to include such substances as ginseng, garlic, fish oils, psyllium, enzymes, glandulars and mixtures of these. A dietary supplement is not for use as a conventional food or as the sole item of a meal or diet.

Phytochemicals are defined as non nutritive plant chemicals having protective or disease preventive properties. There are more than 1000 known phytochemicals, some of which are listed below (Table 1). Plants produce these chemicals which are primarily alkaloids as a means of protecting themselves from environmental pressures and challenges. From a therapeutic perspective some of these plant alkaloids may additionally protect humans against infectious and neoplastic disease processes. These compounds are not essential nutrients and are not required by the human body for sustaining life.

Additionally, the Food and Drug Administration may regulate nutraceuticals as a special category. Should this category be approved by the Obama administration the FDA would set up a premarket approval system for nutraceuticals that is potentially less stringent than that applied to new drugs but significantly more stringent than that applied to dietary supplements. In exchange, companies gaining FDA approval would receive 10 years of market exclusivity. The term nutraceutical would cover dietary supplements, foods or medical foods that 1. possess health benefits, defined as reducing the risk of a disease or health condition, including the management of a disease or health condition or the improvement of health 2. are safe for human consumption in such quantity, and with such frequency, as required to realize such properties. Companies would be required to conduct at least one clinical trial to gain FDA approval.

What is a functional food?

The difference between a food and a drug is that a drug has a defined mechanism of action, binding to a specific receptor in the body with a characterized effect and a food is ingested for nutrition. Functional foods are foods that provide health benefits in addition to basic nutrition, and as such have biological activity with mechanisms of action which are identical to drugs. Therefore, functional foods provide both nutrition as well as benefits normally associated with the ingestion of drug compounds.

Many foods can be considered as functional foods. Examples of functional foods include:

- **Catechins** found in black and green teas which may reduce the risk of cancer
- **Sulforaphane** in broccoli which may reduce the risk of cancer
- Omega 3 fatty acids in fish oils or flaxseed which may reduce the risk of cardiovascular disease and enhance the production of chondroitins in joint fluids
- **Phytochemicals** founds in plants may reduce the risk for cardiovascular and malignant diseases. Phytochemicals include such agents as isoflavones from soy, antioxidants from vegetables and lycopenes from tomatoes
  Yogurt and other fermented dairy products which contain probiotics such a Lactobacillus acidophilus and Bifidobacterium longum which are used to improve gastrointestinal health. Lactic cultures must not only tolerate and pass through the low pH of the stomach but grow and proliferate at physiological levels of bile salts and adhere to intestinal epithelial cells.
- **Lycopene** found in tomatoes which may reduce the risk of cancer of the prostate
- **Soy isoflavones** which may act as either endocrine disruptors or selective estrogen receptor modulators
- **Polyphenolic agents** found in purple grape juice which may reduce the risk of cardiovascular disease
- **Brassica vegetables** in general and broccoli in particular which may protect humans against cancer since they are a rich source of glucosinolates as well as possessing a high content of flavenoids, vitamins and mineral nutrients. The brassica or mustard family of vegetables include broccoli, Brussels sprouts, cabbage, cauliflower, turnips, collards, kale, mustard and Bok choi
- **Oats and oat containing foods** containing the soluble fiber beta glucan which reduce the level of cholesterol
- **Mushrooms, glutamine and antioxidants** may decrease the toxicity of common chemotherapeutic agents
- **The herbal agent huperzine A acts** as a acetyl cholinesterase inhibitor and slows down cognitive decline in the same fashion as the first 4 FDA approved drugs for Alzheimer's – tacrine, donepezil, rivastigmine and galantamine. It is a potent, reversible, selective inhibitor of acetylcholine with similar or higher potency than donepezil.
- **Rhodiola** is an herbal supplement derived from the root of the Rhodioila rosea plant, also known as goldenroot or roseroot. This agent has a direct action on the neuro-endocrine limbic centers including the amygdale and hypothalamus. In so doing it is able to modulate the hypothalamic pituitary adrenal axis against stress induced anxiety.

Table 1 List of representative phytochemicals

Alkaloids

- Caffeine
- Theobromine
- Theophylline

Anthocyanins

- Cyanidin
- Malvidin

Lignans

- Silymarin

Monophenols

- Hydroxytyrosol

Monoterpenes

- Geraniol

- Carotenoids

Beta-Carotene

- Lycopene
- Coumestans

Flavan-3-Ols

Flavonoids

- Epicatechin
- Hesperidin
- Isorhamnetin
- Kaempferol
- Naringin
- Nobiletin
- Proanthocyanidins
- Quercetin
- Resveratrol
- Rutin
- Tangeretin

Hydroxycinnamic Acids

- Chicoric acid
- Coumarin
- Ferulic acid
- Scopoletin

Isoflavones

- Daidzein
- Genistein

- Limonene

Organosulfides

- Allicin
- Glutathione
- Indole-3-Carbinol
- Isothiocyanates
- Sulforaphane

Other Phytochemicals

- Damnacanthal
- Digoxin
- Phytic acid

Phenolic Acids

- Capsaicin
- Ellagic Acid
- Gallic acid
- Rosmarinic acid
- Tannic Acid

Phytosterols

- Beta-Sitosterol

Saponins

Triterpenoids

- Ursolic acid

Xanthophylls

- Astaxanthin
- Beta-Cryptoxanthin

The mechanisms of action for most of these compounds are being worked out in detail. As these functional foods work against selective target receptors such as signal transduction targets leading to alterations in intracellular cytokines and intracellular modulators they have a mechanism of action in the body identical to natural and synthetic drug compounds. Simple overviews of some mechanisms of action are as follows.

1. **Antioxidants** – protection against oxidative damage may reduce the risk of certain types of malignancies. Known phytochemicals with antioxidant activity are allyl

sulfides found in onions, leeks and garlic, carotenoids in fruits and carrots, flavenoids in fruits and vegetables and polyphenols in tea and grapes
2. **Hormonal action** – isoflavones which are found in soy imitate human estrogens and help to reduce menopausal symptoms and osteoporosis
3. **Stimulation of enzymes** – terpenes are found in citrus fruits and cherries and protease inhibitors are found in high concentration in soy and beans
4. **Interference with DNA replication** – saponins found in beans interfere with DNA replication. Capsaicin found in hot peppers protects DNA from carcinogens through the ability to scavenge peroxide radicals as well as OH* radicals
5. **Physical action** – some phytochemicals bind physically to cell walls which may prevent the adhesion of pathogens to human cell walls. Proanthocyanidins provide the anti-adhesion properties of cranberries which may be useful in reducing the risk of urinary tract infections.

There are times when the unique biological compound providing established benefits from a natural product cannot be easily isolated. Biological and environmental samples place great demands on separation and purification techniques. These samples are complicated mixtures of compounds with a variety of physical and chemical properties. Herbal products for example are very complex, often containing hundreds of compounds, and one cannot always be certain which compounds are responsible for the desired therapeutic properties. When the active ingredient in a nutraceutical is not known the possibility of an overdose becomes quite likely.

The US Pharmacopoeia (USP) has established an ingredient verification program for determining whether nutraceutical agents provide concentrations and biological activities as claimed. The USP under this program performs a Good Manufacturing Practices audit as well as a review of the manufacturing and quality control documents and analytical testing from different batches of production. The USP Ingredient Verification Program has the assurance that:

1. the ingredients are consistent in quality from batch to batch. Batch to batch uniformity, which is an assurance that each and every batch conforms to specifications, occurs as long as each step in the Master Manufacturing Record (MMR) is performed and checked. For each lot manufactured, a Batch Production Record must be prepared. The Batch Record provides documentation that every step in the MMR was performed, that the finished dietary supplement meets specifications and that quality control personnel verify this prior to the product being released for distribution.
2. the ingredients meet label or Certificate of Analysis claims for identity, strength, purity and quality. The cGMP requires confirmation of the identity through examination or testing for every lot. Manufacturers can no longer rely on the raw material supplier's Certificate of Analysis for a particular lot of a dietary ingredient as proof of its authenticity.
3. the ingredients are prepared in accordance with accepted manufacturing processes
4. the ingredients meet requirements for acceptable limits of contamination

As part of the new FDA legislation there is an entire subpart devoted to requirements for the operation of an analytical laboratory. The laboratory facilities must be adequate to perform whatever tests and examinations are necessary to determine if specifications are met for raw materials, materials in progress and finished goods. Thin layer chromatography (TLC) is an excellent means for the qualitative identification of herbals and for purity evaluations. Given that fact that most botanical agents are plant alkaloids TLC plants coated with alumina have

become the preferred screening tool of choice, as botanicals have species specific fingerprints. HPLC is an excellent tool for the quantitative analysis of marker compounds in botanical samples. Selecting a desired phytochemical is an appropriate method of establishing a quantitative analysis for a marker compound. Gas chromatography may be useful for the analysis of volatile marker compounds and residual solvents.

HPLC is the most efficient method for the qualitative and quantitative analysis of many botanical agents, allowing great sensitivity, resolution, reproducibility and speed of analysis under inert conditions. The potency of this technique has been improved by the introduction of new detectors such as diode array detectors allowing detection at several wavelengths and simultaneous identification by UV spectral analysis as well as mass and nuclear magnetic resonance detectors. The reader is referred to the Primer on Column Chromatography available from Dynamic Adsorbents for further discussion regarding the use of HPLC techniques for nutraceutical purification.

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