MONOGRAPH TESTING GENERAL INTEREST ARTICLE FOR THE INGREDIENT, DIETARY SUPPLEMENTS, FOOD & BEVERAGE, ANIMAL HEALTH, AND LOW-RISK PHARMACEUTICAL INDUSTRIES: AN OVERVIEW OF THE BENEFITS AND APPLICATIONS OF MONOGRAPH TESTING

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SUMMARY

This article justifies the use of monograph (compendial method) testing in the raw material (ingredients), dietary supplement, infant formula, food, beverage, adult nutritional, animal nutrition, food fortification, and OTC/low-risk pharmaceuticals industries. A brief overview of different testing possibilities based on product types, GMP (Good Manufacturing Practices) requirements, and industry standards is included. Considerations for choosing an internal or independent laboratory are discussed, and examples of Certificates of Analysis (COAs) produced as a result of monograph testing in different industries are included.

OVERVIEW

What is a monograph?

Monographs are documents that provide methods and guidelines to verify the identity, potency or strength, purity, and performance of raw materials and ingredients in a product. They detail the parameters expected of specific products to ensure federal and industry requirements for various criteria, described below, are met. Basically, monographs are lists of tests provided by credible and industry-recognized sources to properly authenticate and analyze ingredients.

Monographs outline tests for measuring the following criteria:

1. **Identity:** Substance is the material that it claims to be
2. **Strength:** Acceptable ranges for the potency of a product
3. **Contamination:** Limits and ranges of impurities permitted
4. **Performance:** Predict and demonstrate how a product will be released in the human body or in some cases animals

Monograph testing is used in a variety of industries, including raw material suppliers, food, beverages, dietary supplements, nutritional products, animal nutrition, and Over The Counter products (OTCs)/low-risk pharmaceuticals.

What are the benefits of monograph testing?

There are many benefits to monograph testing, no matter the industry, including compliance with legal and industry expectations, like cGMP or the US Food Safety Modernization Act (FSMA). Using monograph testing to ensure products meet label claims also helps avoid costly investigations and recalls while improving brand reputation with respectable and industry-recognized practices. Ultimately, monograph testing is the right thing to do for customers, employees, and stakeholders!

More details about the importance of understanding and undergoing monograph testing are provided below:

- **Compliance:** Meet the US Food and Drug Administration (FDA) recommended standards for product purity, identification, potency, and composition with a validated set of tests. Monograph testing provides reliable, reproducible results with the use of standardized and well-developed methods.
- **Reputation:** A more positive reputation is built around properly tested and verified products and brands that value valid testing. Reputations are questioned when a company does not comply with FDA recommendations.
- **Investigations when (not if) something happens:** When an adverse effect is reported or another situation arises that questions any aspect of a product, it is best to have the tests done beforehand instead of rushing testing after the fact. This provides evidence of safety or allows an investigation into the origin of an issue. It is always best to prevent a costly investigation by testing material before sale.
- **The right thing to do:** Value the consumer's safety and trust; even if you think your product is safe, it is always best to make sure.
- **Other benefits:** Improved product quality and safety assurance, recall prevention, adulteration detection, and legal, insurance, and financial claims prevention.

Where do monographs come from?
Many recognized industry organizations exist that develop, validate, and optimize tests for specific ingredients which are compiled into monographs. These organizations are impartial and are only involved in method development, not product development, meaning their suggested tests are truly scientifically acceptable.

The most common sources for monographs are:
- **USP**—US Pharmacopeia: *usp.org*
- **FCC**—Food Chemicals Codex: *foodchemicalscodex.org*
- **ACS**—American Chemical Society: *pubs.acs.org*
- **CP**—Clinical pharmacology: *clinicalpharmacology-ip.com*
- **EP**—European Pharmacopoeia: *pheur.edqm.eu*
- **JP**—Japanese Pharmacopoeia: *pmda.go.*
- **JECFA**—Joint FAO/WHO Expert Committee on Food Additives: *fao.org*

Legal requirements for monograph testing in different industries:
As an example, the US FDA outlines specific monographs for product release on many categories of ingredients and formulated products:

- **Over the Counter (OTC):** OTC products are grouped by therapeutic category, and each category has an OTC monograph. This is essentially a "rule book" establishing conditions for the OTC to be Generally Recognized as Safe and Effective (GRASE). OTC products do not need FDA pre-market approval, however they must comply with (current) cGMP requirements as outlined in 21 CFR parts 210, 211.
- **Animal nutrition:** The US FDA Center for Veterinary Medicine (CVM) also outlines cGMP requirements for medicated animal feeds including Type A medicated articles (concentrated forms) and Type B and C medicated animal feeds for livestock (beef, pork, poultry, aquatic) as well as companion animals (dogs, cats, etc.).
- **Raw materials:** Raw materials do not require monograph testing in some cases, however, it is good practice to test materials for safety, purity, and efficacy verification.
- **Dietary supplements:** In the dietary supplement industry, it is the manufactures responsibility to test for purity, ID, quality, strength, and composition of their products. The FDA does not pre-approve products for sale, however they are involved with post-market enforcement with guidance outlined in 21 CFR part 111. So, monograph testing is important to have beforehand when (not if) a problem arises with a product in the market.

Amongst all industries, monograph testing provides a validated, standardized approach to test ingredient, dosage, and strength claims, as well as screen for contaminants and other potential issues with unintentional adulteration.

TESTING TYPES, SAMPLES, AND INSTRUMENTS

Specific testing methods and workflows are dependent on the substance, material type, ingredient mix, and processing stage. Testing in a capable and accredited laboratory confirms the quality, purity, absence of contaminants, and compliance with regulations for product release or sale.

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1. **Compendial:** This is just another word for monograph. So, if you see a reference to compendial tests or certifications that means monograph testing is used. As described above, compendial testing includes validated, standardized specifications, test methods for a specific product, compound, substance, etc. There are many advantages to compendial testing, a main one being avoidance of validating tests on your own. Additionally, regulatory agencies are familiar with, and typically expect, the outcomes of compendial testing, making the approval and review processes (or any post-distribution follow-ups) more straightforward.
2. **Non-compendial:** This is any testing not specified in a monograph, including in house and independent lab procedures. While these testing methods are usually scientifically reliable, they do not have the same rigorous validation workflow to ensure between-lab reproducibility. Benefits of non-compendial testing includes customized approaches to unique ingredients, flexible problem-solving, and more identification possibilities.

Types of samples:

Many industries use compendial, or monograph testing, so there are methods available for foods, beverages, infant formula, dietary supplements, animal food, chemicals, and more. Methods exist to investigate most stages of product production, including individual compounds, powders, raw materials, and finished products.

Instrument and test types:

The following are examples of the kinds of tests listed in a monograph. Some products or ingredients will only have one test recommended, while other, more complex samples may have multiple. It is important to evaluate the testing requirements for a product to ensure a chosen lab has the instrumentation, quality accreditations and expertise to perform all steps of the monograph. For each test listed in the monograph, there are procedures, analytical methods, and specific results or acceptable ranges for the ingredient, and often it is recommended to optimize methods using blanks and reference standards prior to testing samples. It should be noted that this list does not describe all possible options, and further research should be conducted to understand the specific tests in a monograph.

- **Thermal and physical tests:**
  - **pH, melting point, boiling point, flash point, density, particle size, appearance color:** These are some examples of straightforward tests to confirm the basic physical properties of a product. They are standard methods that can be performed in most labs, but are rarely the only component of a monograph.
  - **Thermal gravimetric analysis (TGA):** An approach to measure the thermal stability of a product, as well as its volatile components. Samples are heated, and weight change is measured over time.

    - **Differential scanning calorimetry (DSC):** The amount of heat energy necessary to change the temperature of a sample measured over a range of temperatures as the sample is heated. The amount of heat required should be in a similar range as a reference standard and may give indications of phase changes during heating.
- **Elemental analysis:**
    - **Inductively Coupled Plasma with various forms of detection – Optical emission spectrophotometry (ICP-OES) or ICP with mass spectral detection (ICP-MS):** ICP-OES is widely used for inorganic elemental analysis, including nickel, phosphorous, sodium, magnesium, copper, and many more. When plasma excites atoms, they release energy measured as photons; each element has a different emission spectrum, so elemental composition can be accurately quantified often at parts per million (ppm) and in some cases at parts per billion (ppb) levels.
    - **Energy dispersion X-ray analysis (EDXA or EDS):** Electron beams are targeted at the surface of a non-volatile solid material under vacuum, resulting in the emission of X-rays that are measured by a detector. Different elements have different X-ray emissions. This technique is typically used for evaluation of heavier elements, and especially heavy metals. One drawback is that EDXA/EDS is semi-quantitative so can't typically be used for product release testing but useful for identification of contaminants during investigations.
    - **Ion selective electrodes (ISE):** This uses electricity to measure the concentration of an ion. A highly selective membrane is attached to an electrical transducer to measure the activity of an ion, either in solution or solid state.
- **X-ray diffraction:** X-ray diffraction (XRD) is a common, yet complex approach for compound structure elucidation. In most solid materials that are crystalized, x-rays are diffracted by the atoms in the sample. The diffraction pattern can be used to determine the crystal structure of a compound, evaluate macromolecules, and confirm sample quality.
- **Chromatography**
    - **High performance liquid chromatography (HPLC) and gas chromatography (GC):** Chromatography separates compounds based on their weight and affinity to certain small particles in an array of specialty chromatography columns that the lab scientists will select along with instrument conditions for optimal accuracy, sensitivity on analyte(s) of interest for prescribed monograph or client parameters. Bigger compounds move slower, so a detector can measure smaller compounds first. Compounds of different polarity are more attracted to the particle in the instrument and are slowed down, allowing even more separation. Liquid chromatography measures the movement of compounds in a liquid form, while gas chromatography measures gaseous compounds based on volatility but also functional groupings can be elucidated with a variety of preparations methods (direct injection, headspace, solid phase micro extraction, "SPME" fibers, etc). HPLC and GC are typically used for targeted analysis and quantification of marker compounds specific to the product in monograph testing, and are often coupled to a mass spectrometry instrument for more detailed identification at ppm and ppb levels in some cases.
    - **High performance thin layer chromatography (HPTLC):** HPTLC is a modern version of TLC, which is one of the first compound separation techniques. HPTLC starts with a plate coated with a thin layer of very small particles. Instruments apply samples to the bottom and a solvent slowly saturates the plate from the bottom, separating compounds in the sample based on their affinity to the plate. HPTLC produces very reproducible results that are easy to compare for sample authentication.
- **Spectroscopy**
    - **Fourier transform infrared spectroscopy (FT-IR) and near IR (NIR):** IR radiation (or near IR) is passed through a sample and the transmitted radiation that is not absorbed is measured with a detector. The resulting signals are transformed into a user-friendly spectra. Different compounds have different spectra, allowing comparison for identification of compounds and analysis of targeted markers. Most reputable labs have spectral libraries with over 100,000 reference spectra for rapid identification of compounds.

- **Raman spectrometry:** Similar to X-ray diffraction, with Raman spectrometry, a laser is focused on a sample and is scattered based on the sample's chemical composition. The "scattering" is measured as the intensity and wavelength, and different chemical groups have different peak shapes that can be pieced together to identify a compound. Raman spectrometry is a common approach for evaluating contamination and impurity of a sample and has the advantage over FTIR and Near IR in that samples containing water or liquid mixtures can be quickly analyzed.

- **Other approaches**
  - **Enzyme-linked immunosorbent assay (ELISA):** Immunoassays are commonly used to measure large molecules like proteins and antibodies including common allergens. An antibody specific for target molecule is coated on a plate, if present the compound binds to the antibody, and a substrate is used to detect any bound compounds. ELISA assays allow relative quantification and confirmation of larger compounds not possible with other techniques.
  - **Polymerase Chain Reaction (PCR):** PCR is used to amplify specific genes in a sample. This technique can be targeted at a gene only in specific ingredients, or for a subset of genes that are unique to a sample. PCR is most often used for identification of detection of common adulterants.
  - **Microscopy:** Microscopy, including light, fluorescence, polarized light, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) can be used to evaluate the microscopic structure of materials with magnification as high as 100,000X. This is most commonly done for raw materials, as processing procedures typically disrupt the true morphology of a material. Microscopic techniques are also useful to identify foreign materials such as plastic, metal and glass when combined with EDXA/EDS for elemental profiling.

CONSIDERATIONS FOR INTERNAL OR INDEPENDENT LAB:

Monograph testing can be performed in house or by an independent, third-party lab. There are advantages and disadvantages to both, but we will focus on the features of an independent testing lab.

Internal

Internal lab testing provides options for companies to factor in routine investigations and approaches into their production times, without having to rely on another company's testing speed. This can improve the consistency of testing and product development as well as shorten the time for raw material acceptance and/or product release. However, it is important that internal testing is highly standardized and reliable, with trained scientists performing tests according to standardized procedures and a robust quality management system (QMS). Internal testing has the benefit of deep knowledge of the product, but that also introduces heavy bias in the interpretation of results and outcomes.

Independent

Independent labs are staffed with interdisciplinary scientists, ranging from analytical chemists to immunologists. This means that no matter the product, there will be someone who knows how to test it. This is a huge benefit, especially as problems arise in the testing workflow. Experienced and knowledgeable scientists are well-versed in troubleshooting and resolving issues with methods and instruments quickly, ensuring reliable and timely results. Additionally, testing in an external lab means product manufactures do not have to search for, train, or pay scientists themselves for monograph testing, not to mention the significant cost to maintain instruments and infrastructure, as well as maintain stocks of costly standard, reagents and lab supplies that independent labs can often purchase in bulk and at significant discounts.

On top of a staff of scientists, there are other benefits and considerations to using a third-party lab for testing. These include:

- Efficient, standardized approaches to data collection and sharing
- Communication with scientific experts and detailed information about procedures
- Little restriction in possible instrumentation and testing abilities
- Fast and predictable turnaround time
- Relationships with monograph developers

Independent Quality Management System Accreditations such as ISO-17025 and in some cases cGMP accreditation for certain forms of testing which reduces the need for auditing of labs by customers and builds confidence in results offered.

WHAT COMES OUT OF MONOGRAPH TESTING?

After monograph testing is complete, a third-party lab will provide you with a few documents. One will be a Certificate of Analysis (COA) which provides an overview of the tests completed, the results, and if the results are within the acceptable range according to the monograph. Additionally, detailed reports of methods, results, and notes will be provided. This open communication about testing procedures is important; trustworthy scientists produce trustworthy results.

EXAMPLES OF COA (certificate of analysis) AND DOCUMENTATION:

1. Single ingredient verification: Taurine

ANALYTICAL REPORT

Eurofins Sample Code: 200-2022-XXXXXXXX
Client Sample Code:
Sample Description: Taurine
Sample Registration Date: 08Jun2022
Condition Upon Receipt: acceptable, non-perishable
Sample Reference:

TK06Y - Taurine by USP (CS)	Reference: Current USP/NF	Completed: 10Jun2022
Parameter	**Result**	**Theoretical**
Assay, dry basis	98.7 %	98.0 - 102.0 %
Identification by IR	Meets Requirements	Meets Requirements
Loss on drying	<0.3 %	NMT 0.3 %
Related Compounds	<0.5 %	NMT 0.5 %
Residue on ignition	<0.6 %	NMT 0.6 %

2. Single ingredient in a liquid matrix: Ethanol in hand sanitizer

ANALYTICAL REPORT

Eurofins Sample Code: 200-2021-XXXXXXXX
Client Sample Code: Vanilla Bean
Sample Description:
Sample Registration Date: 14Jul2021
Condition Upon Receipt: acceptable, non-perishable
Sample Reference:

TK04Y - Alcohol Content	Reference: USP <611> Modified	Completed: 21Jul2021
Parameter	**Result**	**LOQ**
Ethanol	70.0 % v/v	0.1 % v/v

3. Ingredient verification, potency, and impurities investigation: CTC in animal feed

PO# N/A

Attn: Contact Name

Certificate of Analysis

Results shown in this report relate solely to the item submitted for analysis. Any opinion/interpretations expressed on this report are given independent of the laboratory's scope of accreditation. All results are reported on an "As Received" basis unless otherwise stated. Reports shall not be reproduced except in full without written permission of Eurofins SF Analytical Laboratories. All samples were in good condition when received by the laboratory unless otherwise noted. All work done in accordance with Eurofins General Terms and Conditions of Sale. Measurement of uncertainty can be obtained upon request.

Sample Identification: Ambient Month 24 Pallet 2 Box 4

Analysis:	Specification	Result	Method
Appearance	Brown to deep brown granular, not lumpy or moldy; no unpleasant odor-compares to in house standard.	Deep brown granular, not lumpy or moldy; no unpleasant odor-compares to in house standard.	Visual (STP-64)
Identification 1	Deep Purple Color	Deep Purple Color	STP-09 (Sakaguchi rgt)
Identification 2	Retention Time of major peak from sample solution corresponds to that of the standard solution as obtained in the impurities assay	Retention Time of major peak from sample solution corresponds to that of the standard solution as obtained in the impurities assay	HPLC
LOD	NMT 10.0%	8.6%	STP-67
CTC Potency			
Replicate 1	18.7%-25.3%	19.6%	AOAC 957.23/967.39[1]
Replicate 2	18.7%-25.3%	19.3%	AOAC 957.23/967.39[1]
Replicate 3	18.7%-25.3%	19.5%	AOAC 957.23/967.39[1]
Average of Replicates	18.7%-25.3%	19.5%	AOAC 957.23/967.39[1]
TC	NMT 8.0%	1.3%	HPLC[2]
EPICTC (4&6)	NMT 6.0%	1.4%	HPLC[2]
Impurity A (ADTC)	NMT 1.5%	0.7%	HPLC[2]
Impurity B (ADCTC)	NMT 0.6%	0.2%	HPLC[2]
Single Unidentified Impurity	NMT 0.2%	< 0.1%	HPLC[2]
TOTAL IMPURITIES	NMT 2.3%	1.0%	HPLC[2]

[1]Testing performed by Eurofins SF GMP Horsham.
[2]HPLC Method as described in section H-7.4 of CTC Feed Grade

CONCLUSIONS:

Monograph testing is an important step before putting a product on the market. No matter the industry, using standardized methods to test composition and purity improves the safety, purity, potency, consistency and reputation of a product and brand. Trusting an independent lab to perform monograph testing provides non-biased results, however it is important to carefully choose a lab with open communication, experienced scientists, reasonable turnaround times that is convenient to work with. Monograph testing may not be legally required for all industries, but is the industry standard for producing the best product possible.

Let's find your solution. Visit our website at ***eurofinsus.com/monograph***.