Complexity of Dietary Supplement Identity and Purity Testing - Contract Testing Laboratory Perspective

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Outline

- Complexity of Dietary ingredients
- Contract testing of Ingredients
- Reasonably anticipated contaminants
- Case studies of Unknown Unknowns
- Final Takeaway
Dietary Ingredients are Complex

- Sourced globally
- Lots are often mixtures of heterogeneous materials from different sources
- Naturally sourced materials are inconsistent in composition
- Different botanical extraction techniques produce different chemical compositions even if the same source material is utilized
- End users often don’t have a direct connection to the ingredients they source
- Process flow diagrams are often oversimplified and unclear
- Demand and supply economics impact product availability and quality
Contract Testing of Ingredients is Demanding

Contract Research Organization/Contract Testing Lab

- Often clients know less about their ingredients than the CRO does
- An examination of the production flow chart and product specifications/COA is essential BEFORE testing starts
- Methods are often modified to meet unique matrix issues which require additional performance verification
- Undeclared matrix components can negatively impact testing
- Testing ingredients requires continuous education and awareness of emerging issues
- It is impossible to guarantee that any given test will work for all matrices
“As we know, there are known knowns. There are things we know we know. We also know there are known unknowns. That is to say, we know there are some things we do not know. But there are also unknown unknowns — the ones we don't know we don't know,”

Donald Rumsfeld
But First a Quick Review of 21 CFR part 111
21 CFR part 111 RACs

Reasonably Anticipated Contaminants

- 21 CFR 111.70(b)(3) You must establish limits on those types of contamination that may adulterate or may lead to adulteration of the finished batch of the dietary supplement to ensure the quality of the dietary supplement.

- 21 CFR 111.70(c)(2) You must provide adequate documentation of your basis for why meeting the in-process specifications, in combination with meeting component specifications, will help ensure that the specifications are met for the identity, purity, strength, and composition of the dietary supplements and for limits on those types of contamination that may adulterate or may lead to adulteration of the finished batch of the dietary supplement;…
Reasonably Anticipated Contaminants

Commonly Known Examples (*known knowns* and *unknown knowns*)

- Pesticides (residual in soil, applied, overspray)
- Toxic elements (soil, processing, catalysts, foliage dusts, etc.)
- Natural toxins (PA’s, aflatoxins, toxins from other crops)
- Microbial contamination
- Solvent residues (from synthesis or extraction)
- Synthesis byproducts (side reactions, catalysts, chiral impurities, etc.)
- Light and heavy filth (soil, excreta, insect fragments, other FOM)
- Degradation products (oxidative degradation, etc.)
Many RACs are common sense and can be tested for directly

USP, AOAC, and many other standard-setting organizations provide testing protocols for known RACs

Your understanding of the ingredient, how it is produced and where, what the market conditions are, and use history will dictate the RACs which must be screened for

However, what about the unknown unknowns?
Case Studies of Unknown Unknowns

Cast a wide net
Botanical Extract Solvent Residue Screen

**Root Extract**
- COA states ethanol solvent extract
- Submitted for ethanol limit testing
- Lab ran standard USP<467> panel
- EtOH limit passed
- Cyclohexane and ethyl acetate found in sample (unknown unknowns)
- COA and process flowchart do not indicate the use of these solvents
- Targeted residue screen would have failed to detect these solvents

Courtesy of Flora Research Laboratories, LLC
Other Undeclared Solvent Residues

All in products claimed to be ethanol extracts

- Chloroform
- Methylene chloride
- Ethylene dichloride
- Methanol
- Isopropanol
21 CFR part 111 Identity Testing

AT LEAST ONE IDENTITY TEST

- 21 CFR 111.75(a)(1)(i) Conduct at least one appropriate test or examination to verify the identity of any component that is a dietary ingredient, unless you petition the agency under paragraph (a)(1)(ii) of this section and the agency exempts you from such testing;

- Note that the language states “at least one appropriate test or examination to verify the identity” and not “one test”

- Sometimes, one test is not enough, and novel adulteration can evade detection if you rely on a single test
Cross Contamination in Holding/Shipping

**Hyssop**

- Sample failed ID due to uncharacteristic band (unknown unknown) but **passed by microscopy**
- Subsequent analysis showed band to be eugenol
- Ingredient was shipped with cloves in closed container
- Combining the testing methods helped detect contamination that histological examination alone would miss.

Courtesy of Flora Research Laboratories, LLC
Other Volatile Oil Cross Contamination

- Carvacrol detected from storage next to oregano leaf
- Thymol detected from storage next to thyme leaf
- Volatiles from storage adjacent to valerian root powder
- These issues can occur throughout the supply chain beyond the end manufacturer’s control
Foreign Matter – Why HPTLC is not enough

- Foreign matter in parsley at significant levels which does not appear in the HPTLC profile (which was consistent)
- Sand and atypical trichomes
- While some FOM is acceptable, material in great excess is characteristic of poor GAP or gross adulteration
Oregano Leaf Gross Adulteration

- Sample passed HPTLC identity testing
- Oregano is sporadically adulterated with numerous other botanical materials such as sumac and olive leaf
- Features in images E & F are characteristic but abundant stellate trichomes featured above are from an adulterant
- Again, reliance on a single contaminant or adulteration test is not adequate for botanical raw materials

Courtesy of Flora Research Laboratories, LLC
Comfrey Leaf?

GCMS showed menthol, isomenthone and carvone

Product is a tea blend of comfrey leaf, peppermint leaf and spearmint leaf

Courtesy of Flora Research Laboratories, LLC
Bulking Resin with Wheat Starch

Does not appear on HPTLC profile which passed

Wheat is a major allergen
Clandestine Adulteration

Creative Bad Actors Continuously Innovate
Clandestine Adulteration

Case Study

- Some bad actors add APIs or analogues of APIs to their ingredient to enhance its effect.
- This is done to create the impression that their ingredient is higher quality and thus more effective.
- USP<2251> addresses this adulteration scheme with a focus on PDE-5 inhibitors. Hopefully, we will be adding weight loss, blood sugar support, performance enhancement and sleep/relaxation classes to this chapter in the future.
- The highly sophisticated schemes often involve using analogue drugs to evade detection by labs using targeted screening panels.
U-Dream Sleep Aid

Was an Amazon Best Seller and licensed NHP claimed to be all-natural
Except there is the bromine compound

Brominated organic compounds are a red flag for a dietary supplement
Structure of Analogue

Brominated analogue of Zopiclone which shifts the mass and evades targeted screening

Figure 4. Structure of zopiclone (A) and proposed structure of the suspected brominated analogue found in the product (B).
Phytoforensic Approach

This is not plug and play testing
A year after class I recalls, we found many products still available with steroids present (see JAMA Letters)
Detergent additive significantly boosted apparent chondroitin levels

This is why you should perform ALL monograph tests in the USP not just some!
Targeted screening for contaminants is an important step but it is not the only step when working with a global supply chain and highly volatile market. Whenever demand exceeds supply, bad actors will develop sophisticated techniques to evade detection using standard methods. If a narrow-focused lens is used for contaminant testing, important and sometimes dangerous adulterants may be missed. It is imperative that the CRO have adequate expertise, experience and connectivity to the industry to help mitigate the risk of releasing adulterated materials (unknown unknowns).
Thank You

The standard of trust