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

James Kababick

Botanical Dietary Supplements and Herbal Medicines Expert Committee Member

April 21, 2022

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

The “Rumsfeld” Classification of Impurities

“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)

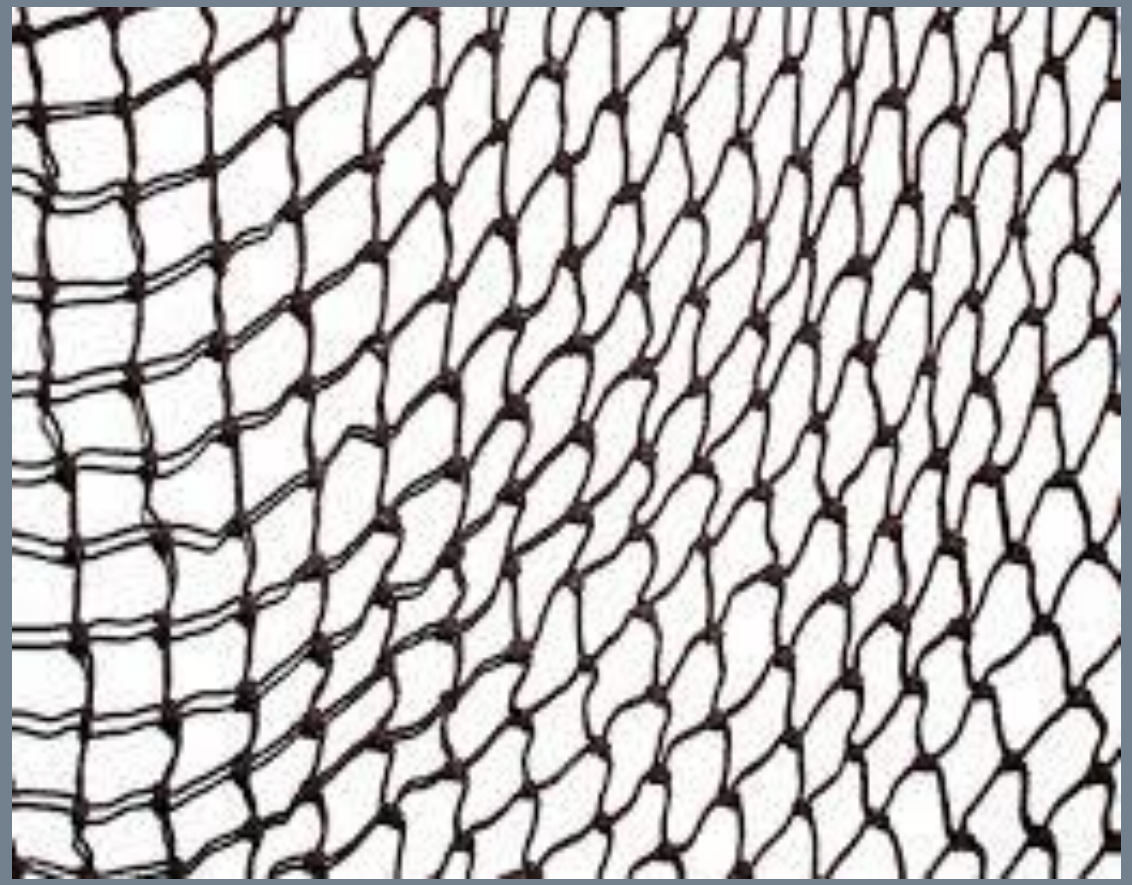
- Pesticides (residual in soil, applied, overspray)
- Toxic elements (soil, processing, catalysts, foliage dusts, etc.)
- Natural toxins (PA's, aflatoxins, toxins from other crops)
- Microbial contamination
- Solvent residues (from synthesis or extraction)
- Synthesis byproducts (side reactions, catalysts,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

RACs

- 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

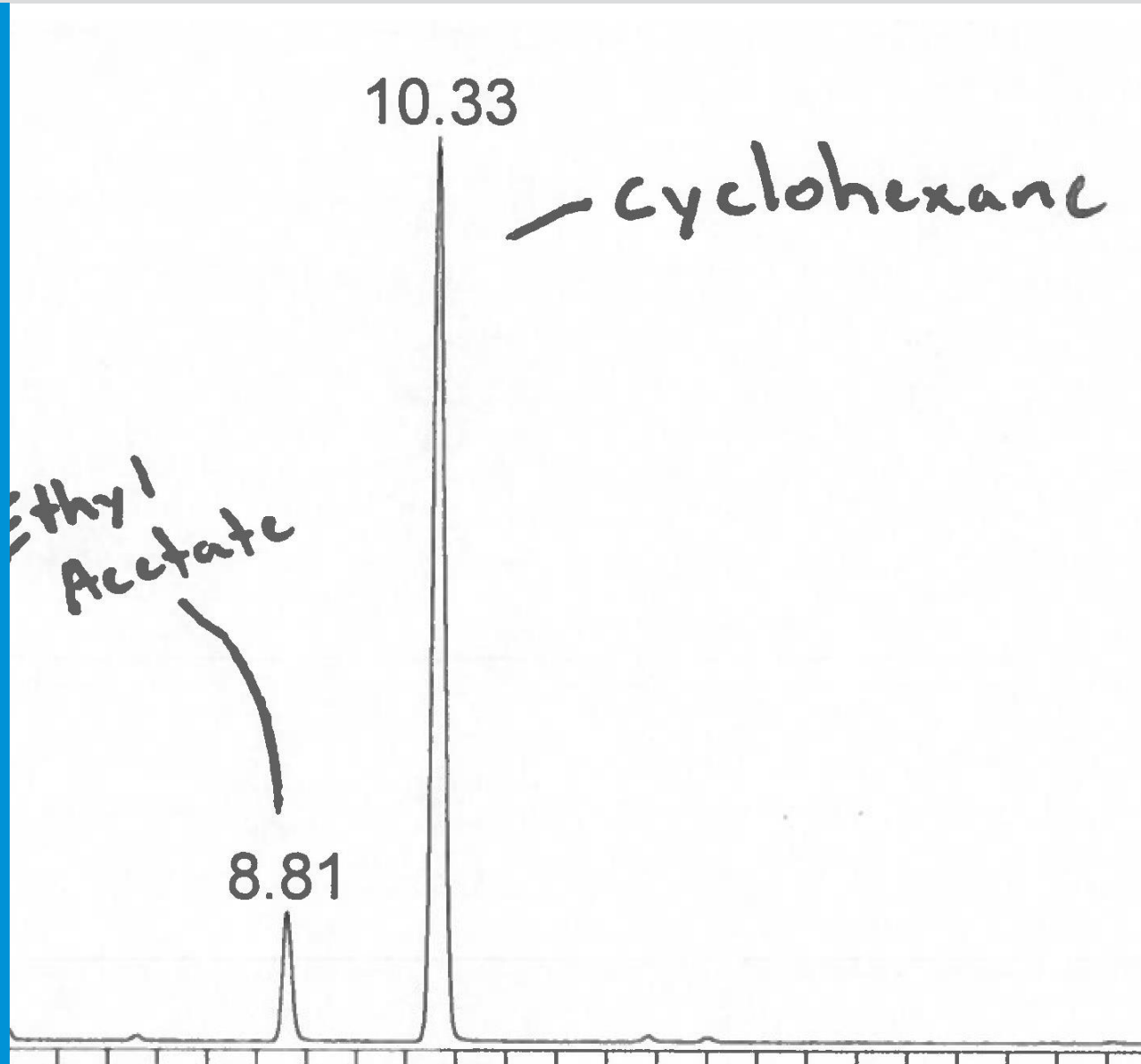
Cast a wide net



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

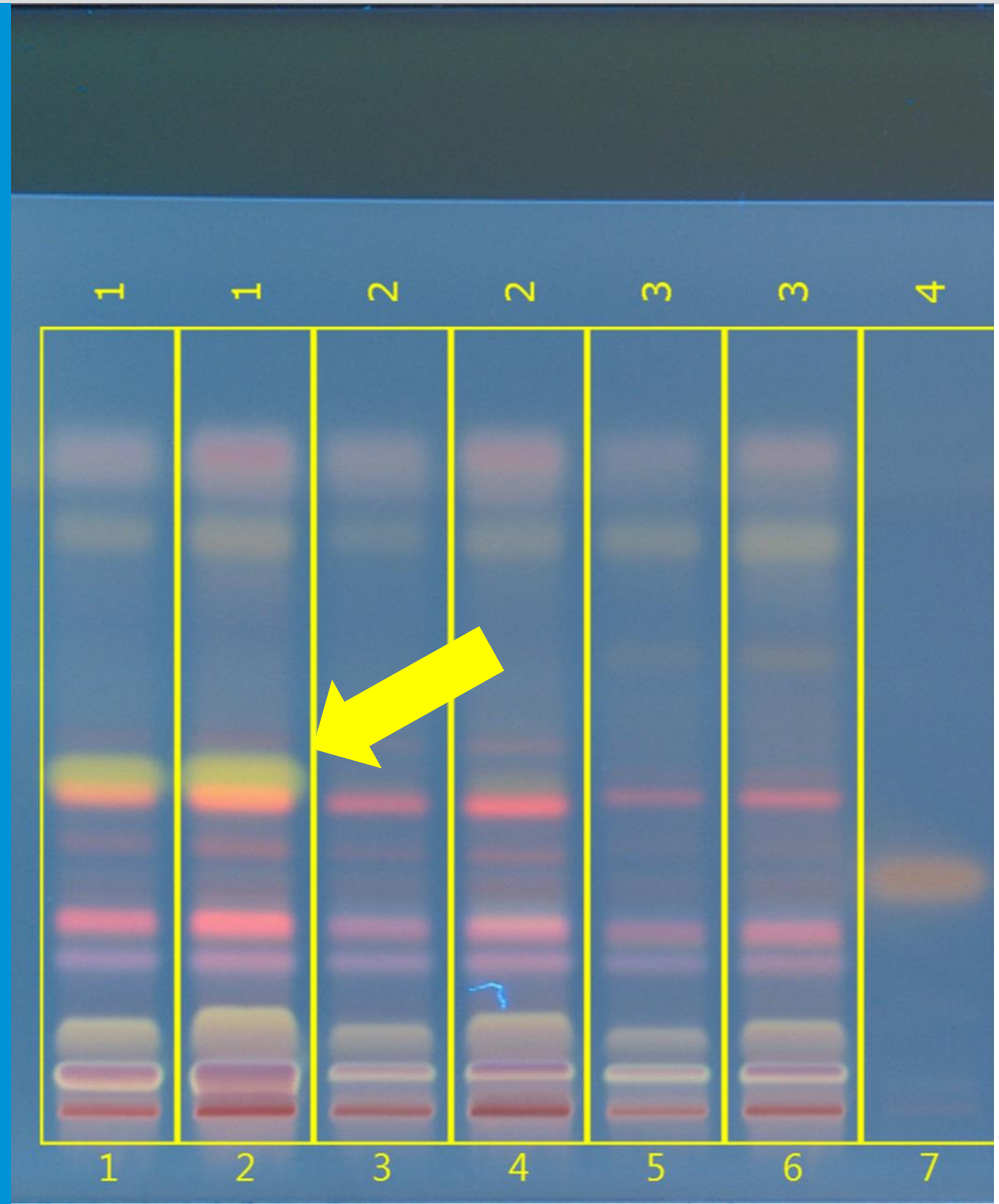
AT LEAST ONE IDENTITY TEST

- 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.



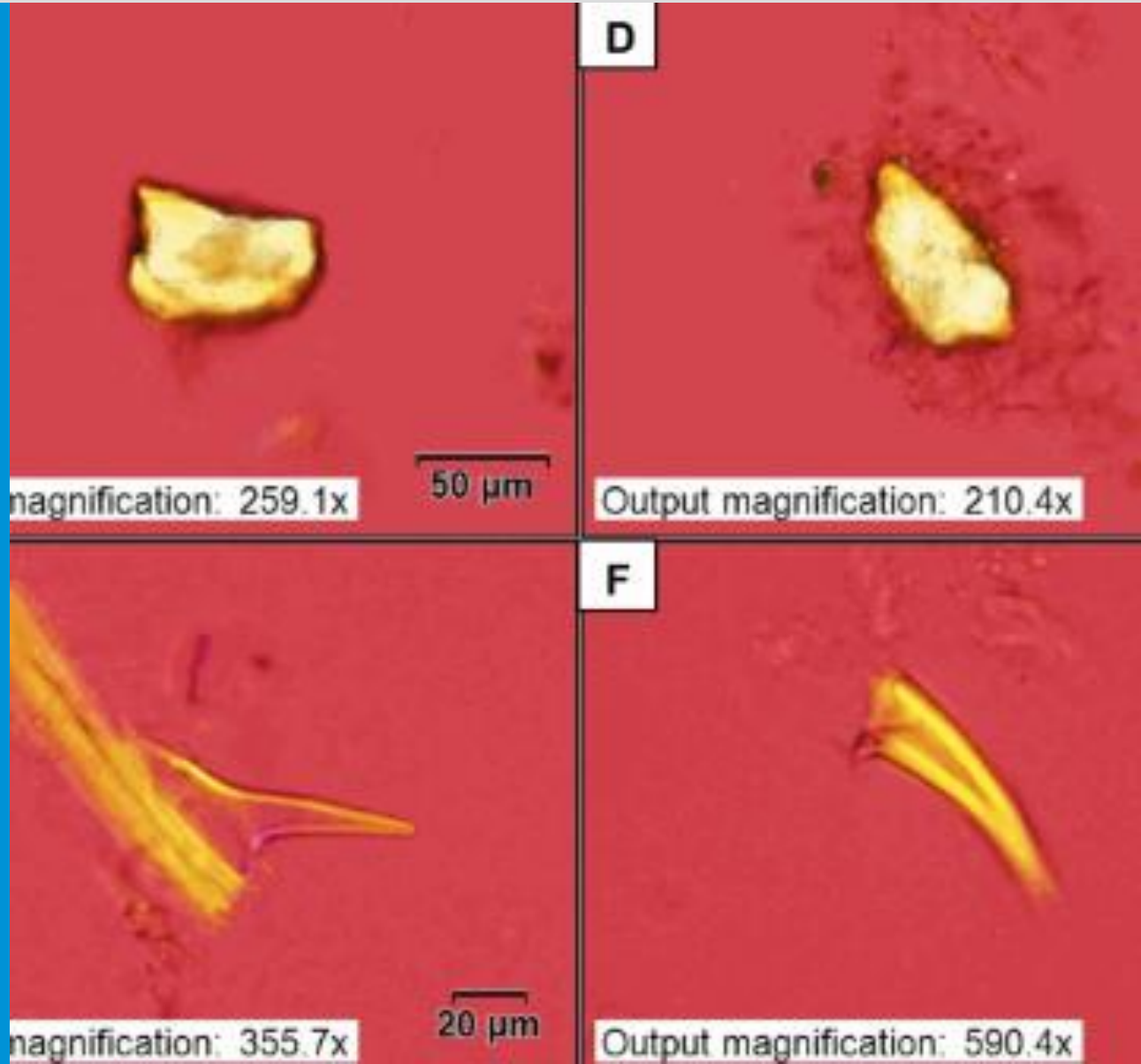
Courtesy of Flora Research Laboratories, LLC

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

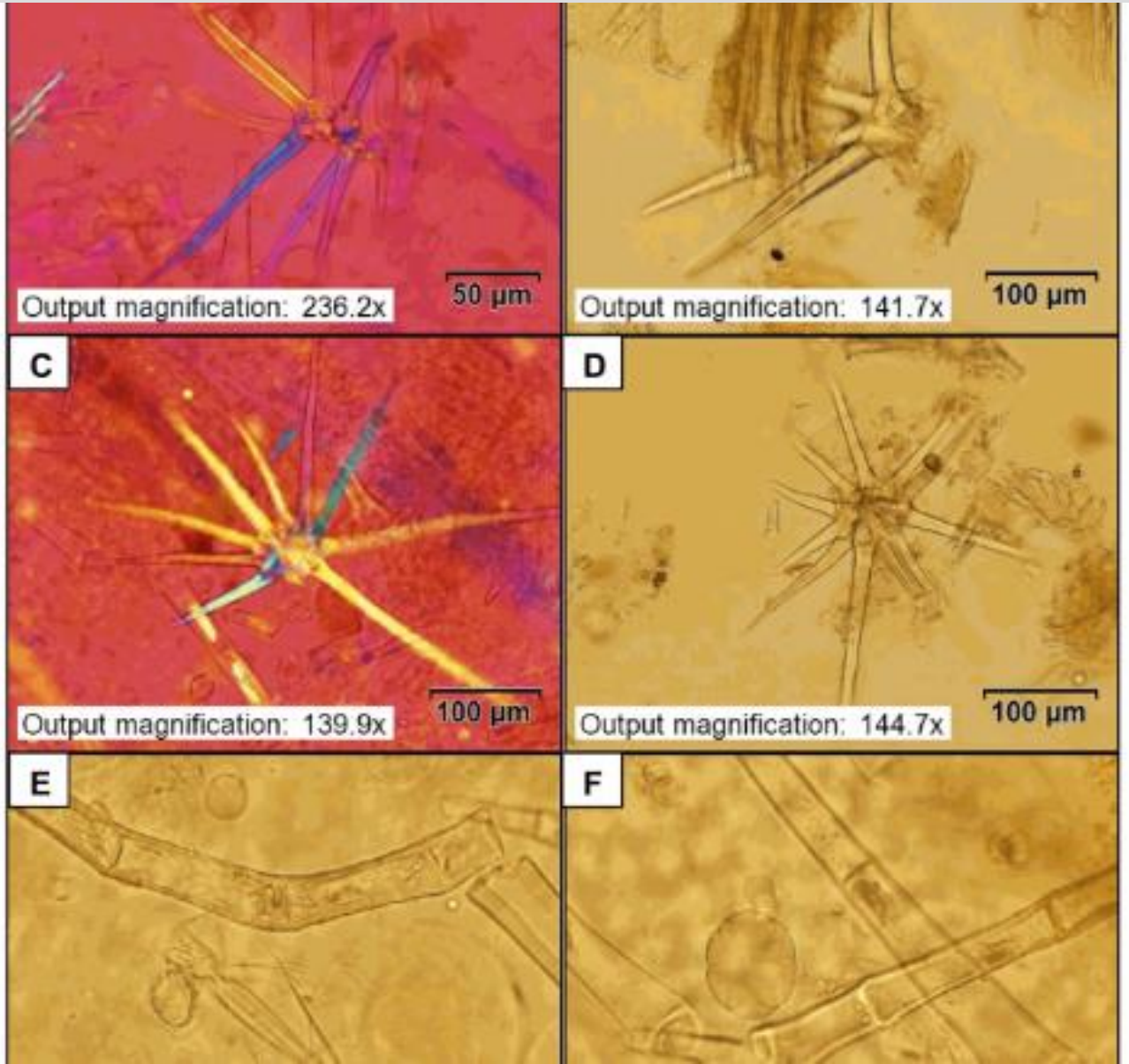
- Foreign matter in parsley at significant levels which does not appear in the HPTLC profile (which was consistent)
- Sand and atypical trichomes
- While some FOM is acceptable, material in great excess is characteristic of poor GAP or gross adulteration



Courtesy of Flora Research Laboratories, LLC

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

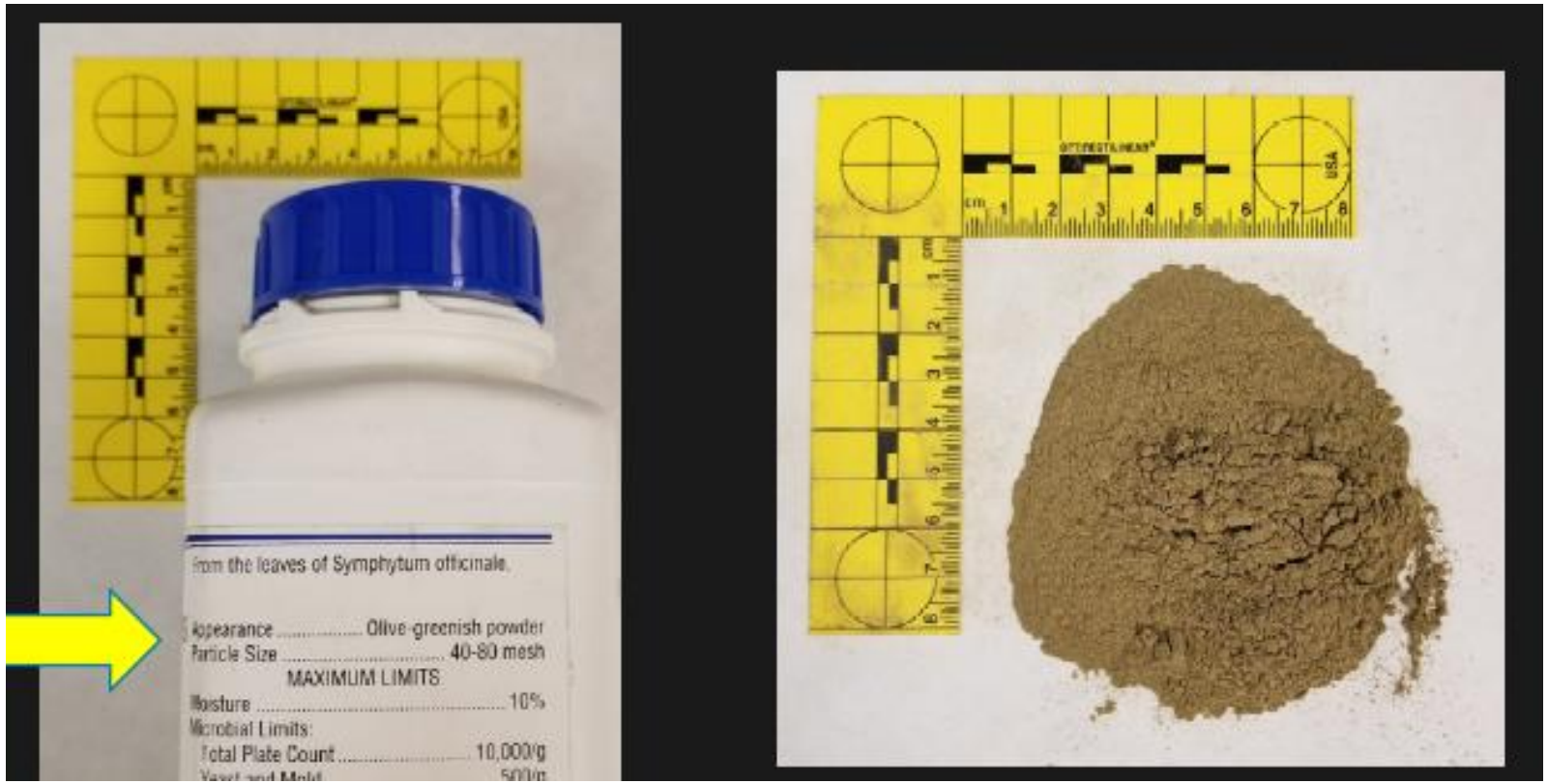


Courtesy of Flora Research Laboratories, LLC

Comfrey Leaf?

GCMS showed menthol, isomenthone and carvone

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

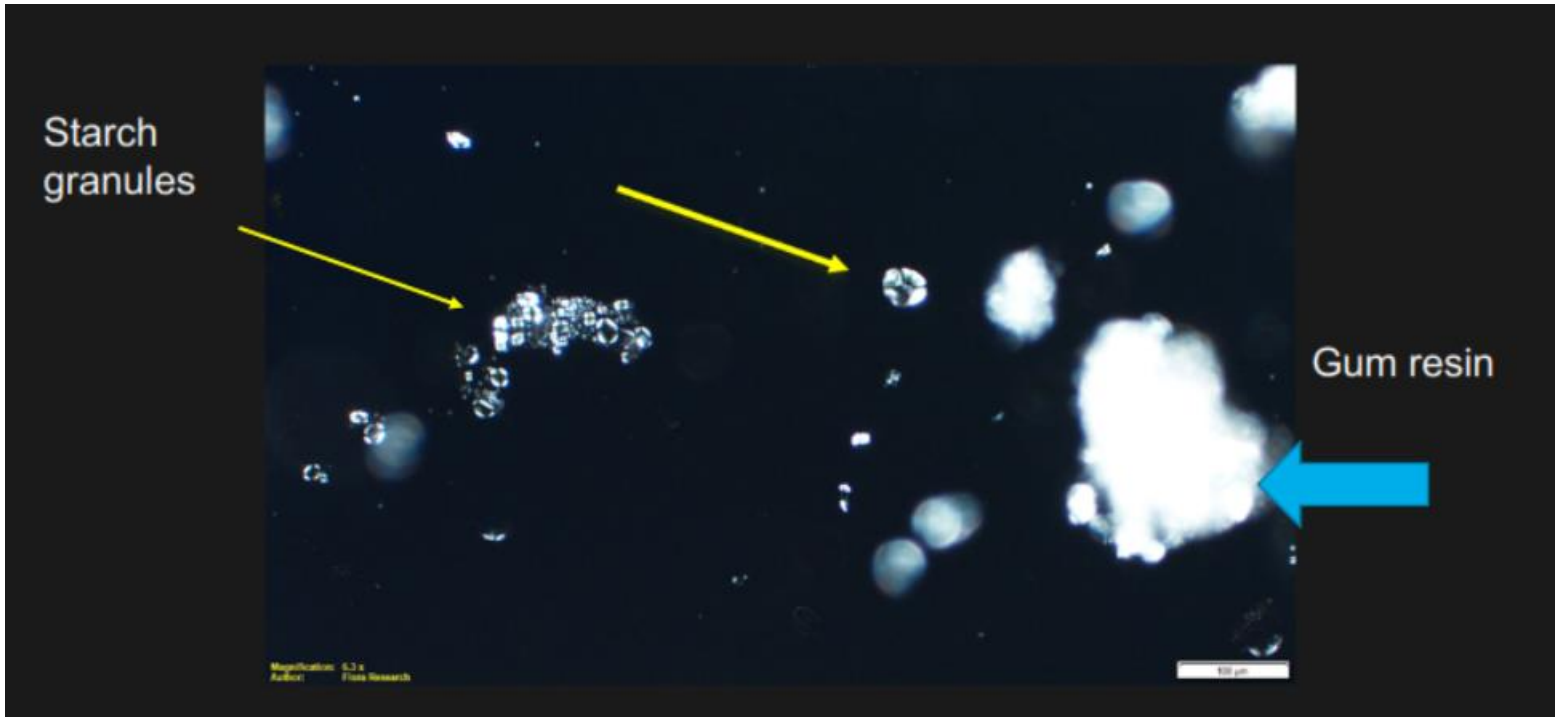


Courtesy of Flora Research Laboratories, LLC

Bulking Resin with Wheat Starch

Does not appear on HPTLC profile which passed

Wheat is a major allergen



Courtesy of Flora Research Laboratories, LLC

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

Journal of Natural Health Product Research
2020, Vol. 2, Iss. 1, pp. 1–12.
NHPPublications.com

Journal of Natural Health Product Research
NHP PUBLICATIONS

Check for updates

ORIGINAL RESEARCH OPEN ACCESS

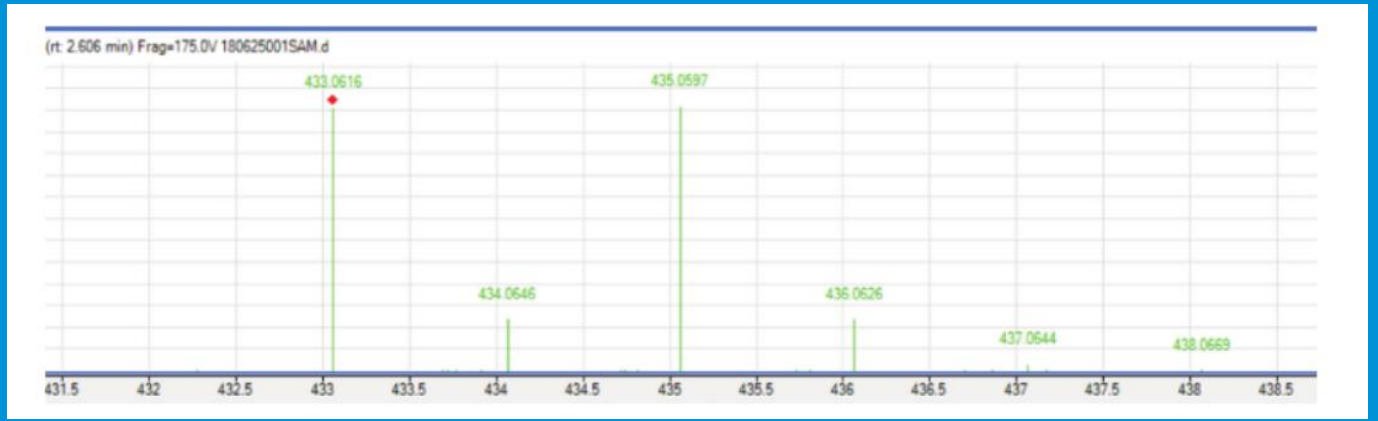
Detection of Undeclared Halogen Substituted Drug Compound in a Natural Health Product

Paula N. Brown, PhD[1,2*], Michael Chan, PhD[1], Chuck Chang, BSc[3], Sheena Kuo, BSc[3], Yoon Seok Roh, BSc[3], James Neal-Kababick[4], Mary L. Hardy, MD[5]

[1]Centre for Applied Research & Innovation, British Columbia Institute of Technology, Burnaby, BC, Canada, V5G3H2
[2]Department of Biology, University of British Columbia, Kelowna, BC, Canada
[3]Isura, 501-3292 Production Way, Burnaby, BC, V5A4R4
[4]Flora Research Laboratories LLC, 1000 SE M Street Unit B, Grants Pass, Oregon, USA, 97526
[5]Academy of Integrative Health & Medicine, 6919 La Jolla Blvd., La Jolla, CA 92037
*Corresponding Author: Paula_Brown@bcit.ca

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

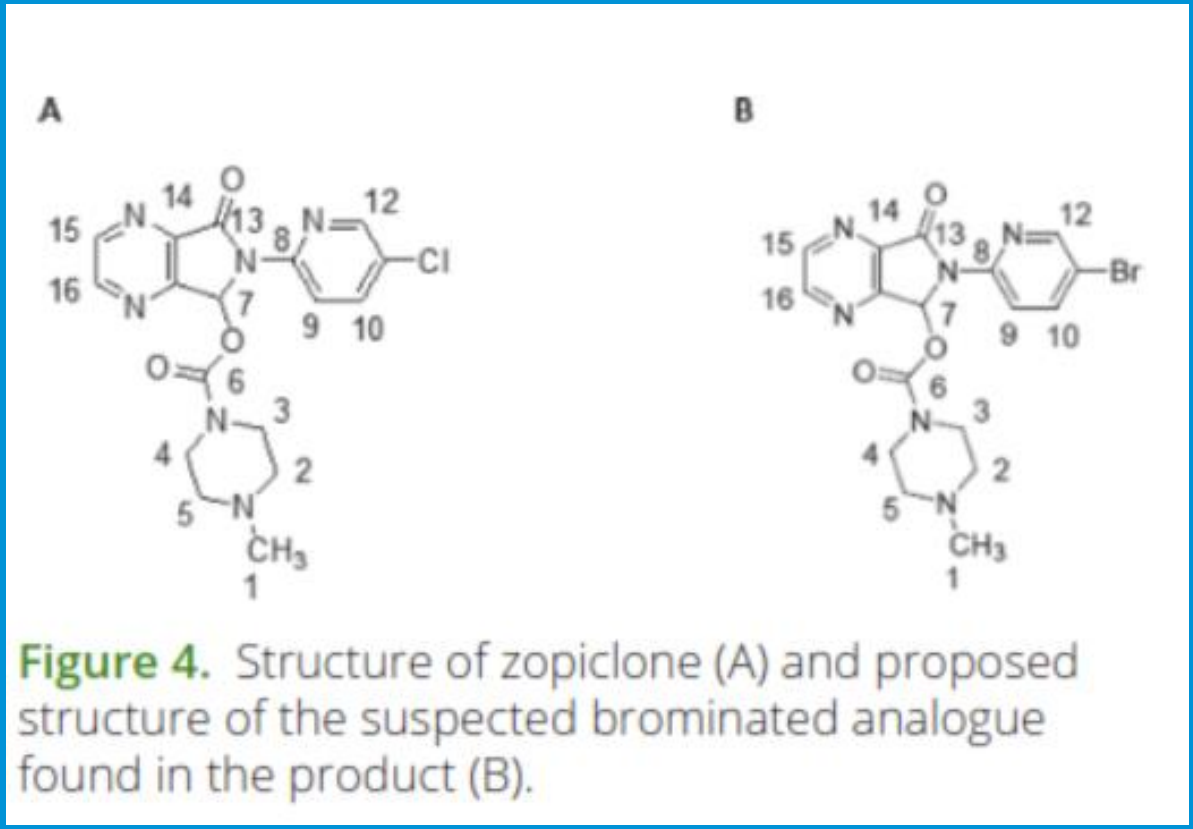


Figure 4. Structure of zopiclone (A) and proposed structure of the suspected brominated analogue found in the product (B).

Phytoforensic Approach

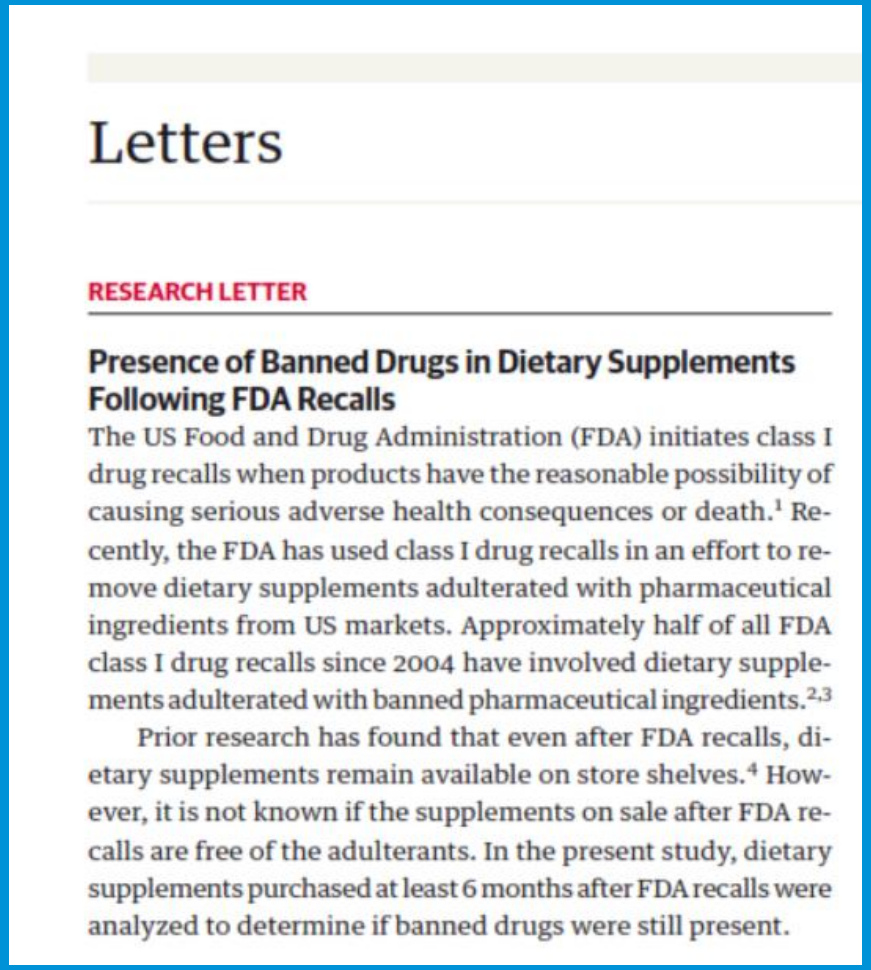
This is not plug and play testing

Table 4. Comparison of major *m/z* for zopiclone reported in Massbank [20], theoretical *m/z* for a zopiclone compound with its chlorine replaced with a bromine atom and observed *m/z* observed from the MS/MS analysis of the 433 *m/z* product ion in the U-Dream product.

m/z (MassBank EQ364303)		Theoretical *m/z* Cl replaced with Br		*m/z* observed in U-Dream product
m/z	Annotation	*m/z*	Annotation	*m/z*
98.0835	C5H10N2+	98.0835	C5H10N2+	98.0843
99.0917	C5H11N2+	99.0917	C5H11N2+	99.0921
111.9949	C5H3ClN+	155.94438	C5H3BrN+	155.9446
139.0059	C6H4ClN2+	182.95538	C6H4BrN2+	182.9550
143.0816	C6H11N2O2+	143.0816	C6H11N2O2+	143.0815
217.1085	C11H13N4O+	217.1085	C11H13N4O+	217.1081
245.0226	C11H6ClN4O+	288.97208	C11H6BrN4O+	288.9713
247.0381	C11H8ClN4O+	290.98758	C11H8BrN4O+	290.9870
263.0332	C11H8ClN4O2+	306.98268	C11H8BrN4O2+	306.98201
277.0487	C12H10ClN4O2+	320.99818	C12H10BrN4O2+	320.9980
345.1227	C16H18ClN6O+	389.07218	C16H18BrN6O+	389.0717

Steroids in recalled products

A year after class I recalls, we found many products still available with steroids present (see JAMA Letters)



Letters

RESEARCH LETTER

Presence of Banned Drugs in Dietary Supplements Following FDA Recalls

The US Food and Drug Administration (FDA) initiates class I drug recalls when products have the reasonable possibility of causing serious adverse health consequences or death.[1] Recently, the FDA has used class I drug recalls in an effort to remove dietary supplements adulterated with pharmaceutical ingredients from US markets. Approximately half of all FDA class I drug recalls since 2004 have involved dietary supplements adulterated with banned pharmaceutical ingredients.[2,3]

Prior research has found that even after FDA recalls, dietary supplements remain available on store shelves.[4] However, it is not known if the supplements on sale after FDA recalls are free of the adulterants. In the present study, dietary supplements purchased at least 6 months after FDA recalls were analyzed to determine if banned drugs were still present.

Spoofing Chondroitin CPC Titration

Detergent additive significantly boosted apparent chondroitin levels

DIETARY SUPPLEMENTS

Electrophoretic Separation of Alginic Sodium Diester and Sodium Hexametaphosphate in Chondroitin Sulfate that Interfere with the Cetylpyridinium Chloride Titration Assay

WEIGUO ZHANG
Synutra Research Laboratory, 2275 Research Blvd, Rockville, MD 20850
GABRIEL GIANCASPRO and KRISTIE M. ADAMS
United States Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852
JAMES NEAL-KABABICK
Flora Research Laboratories, 1000 SE M St, Unit B, Grants Pass, OR 97526
JANA HILDRETH
Synutra Research Laboratory, 2275 Research Blvd, Rockville, MD
AISHAN LI
Meitek Technology (Qingdao) Co., Ltd, Jiaonan City, Qingdao, People's Republic of China
MARK C. ROMAN[1]
Tampa Bay Analytical Research, 13130 56th Court, Suite 606, Clearwater, FL 33760
JOSEPH M. BETZ
National Institutes of Health, Office of Dietary Supplements, 6100 Executive Blvd, Bethesda, MD 20892

- This is why you should perform ALL monograph tests in the USP not just some!

Takeaway

Unknown unknowns & CRO Testing

- 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