

2009 TTB Expo Presentation

Laboratory Analysis of Limited Ingredients, Prohibited Ingredients and Allergens

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Overview

- Background
- Prohibited Ingredients
- Limited Ingredients
- Miscellaneous
 - Pesticides
 - Trace Metals Analysis
 - Ethyl Carbamate
- Allergens
 - Background and regulations
 - Detection in alcoholic beverages

Background

- On Jan 24 2003, Homeland Security Act established TTB
- Formerly associated with ATF
- TTB remains in Treasury Department
- ATF, now called ATFE, was transferred to the Justice Department

TTB's Mission

- To collect excise tax, to ensure products are labeled and marketed according to law, to protect the consumer, and to promote voluntary compliance

TTB Duties

- Responsible for ensuring compliance with the Federal Alcohol Administration Act (FAA Act) and Internal Revenue Code (IRC)
- Govern, amongst other things, the labeling of wines, distilled spirits, and malt beverages in interstate and foreign commerce
- SSD provides comprehensive technical support to all TTB programs; SSD accomplishes its mission through four laboratories

Scientific Services Division Laboratories

- Beverage Alcohol Laboratory
 - Beltsville, MD
- Nonbeverage Products Laboratory
 - Beltsville, MD
- Tobacco Laboratory
 - Beltsville, MD
- Compliance Laboratory
 - Walnut Creek, CA

Formally known as the Alcohol
and Tobacco Laboratory when
associated with ATF

Prohibited Ingredients

Prohibited Ingredients

- FDA establishes if an ingredient is prohibited for use in a food and/or beverage
- In 1987, predecessor agency (ATF) entered MOU with FDA
- TTB will consult with FDA regarding the necessity to label ingredients that pose a public threat
- Regulations do not require a comprehensive list of ingredients
- Examples of prohibited substances that may be associated with alcoholic beverages:
 - Thujone (21 CFR 172.510)
 - Coumarin (21 CFR 189.130)
 - Calamus and its derivatives (21 CFR 189.110)
 - Tin-coated lead foil capsules for wine bottles (21 CFR 189.301)
 - Non-certified synthetic dyes
 - Stevioside

Prohibited Ingredients (Continued)

Thujone Analysis in Distilled Spirits:

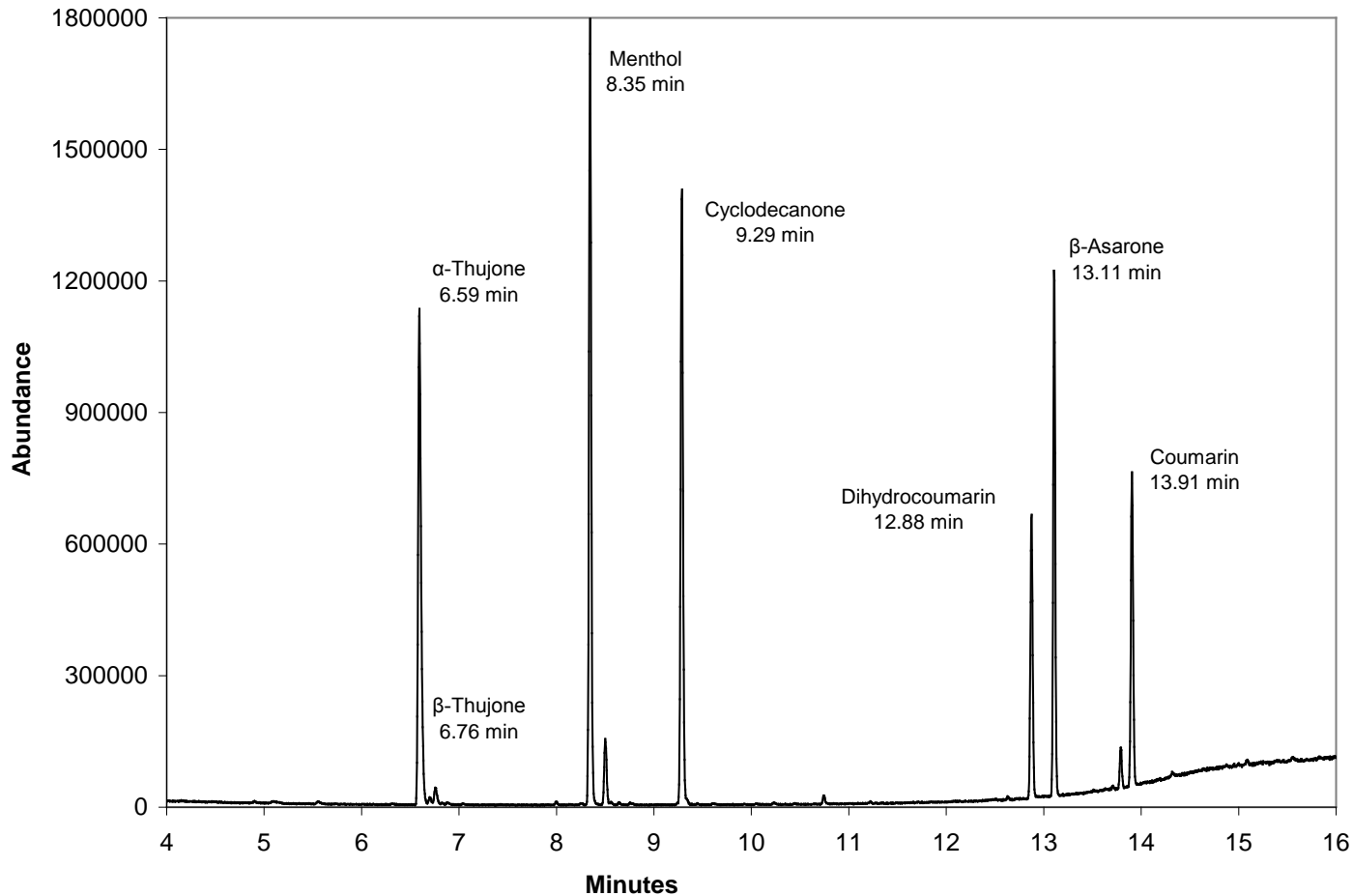
- BAL has received an increased number of absinthe type products since the publication of IC 2007-5 (Use of the Term Absinthe for Distilled Spirits)
- Monoterpenoid compound consisting of two isomers: alpha and beta
- Found in artemesia (wormwood), white cedar, oak moss, tansy, yarrow
- Developed a GC/MS screening method:
 - Scan mode
 - Capillary DB-Wax column, 0.25mm, 30m, 0.25um
 - Menthol as the internal standard, use alpha form for quantitation
 - LOD = 0.3 ppm, LOQ = 1 ppm
- FDA regulation cites JAOAC 19, 120 (1936), with a LOD of 10 ppm
- Samples with thujone levels lower than 10 ppm are considered thujone-free

Prohibited Ingredients (Continued)

- Combined Thujone, Coumarin and beta-Asarone
GC/MS Method:
 - Coumarin is an active ingredient of buffalo grass, tonka beans, sweet woodruff
 - beta-Asarone is an active ingredient of calamus
 - The goal was to combine these analytes into one GC/MS method
 - Menthol is the current internal standard for thujone (some samples contain menthol as an ingredient)
 - Cyclodecanone is being investigated as an alternative to menthol

Prohibited Ingredients (Continued)

GC/MS Chromatogram of Thujone, Coumarin, Asarone and Internal Stds

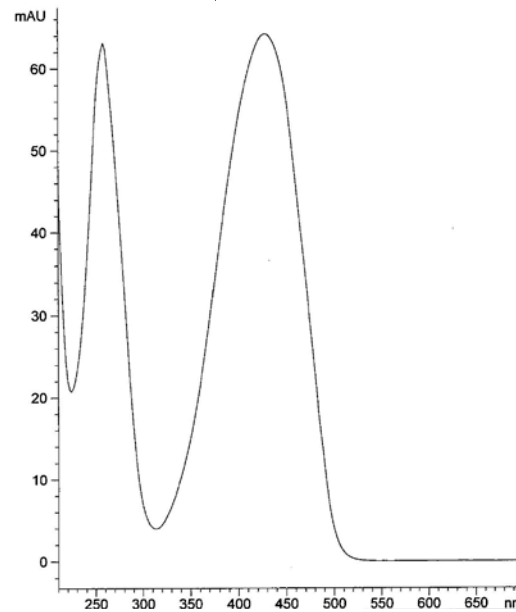
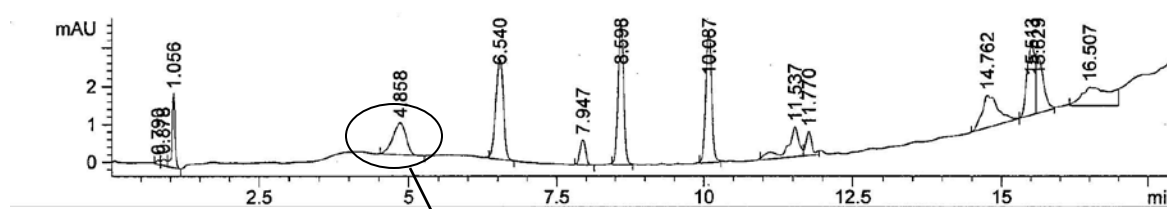


Prohibited Ingredients (Continued)

- Coloring Material:
 - Added to impart a color which is not natural to the product
 - FD&C Certified (allowed) — Yellow #5 (tartrazine) must be declared on the label
 - Noncertified (allowed) — Examples are paprika, saffron, beet extract, caramel
 - Noncertified synthetic dye (prohibited)
 - Developed an HPLC method using DAD, in the process of validating this method, 26 total dyes in library — ODS column, phosphate buffer and methanol as mobile phase, gradient

Prohibited Ingredients (Continued)

HPLC Chromatogram of Synthetic Dyes



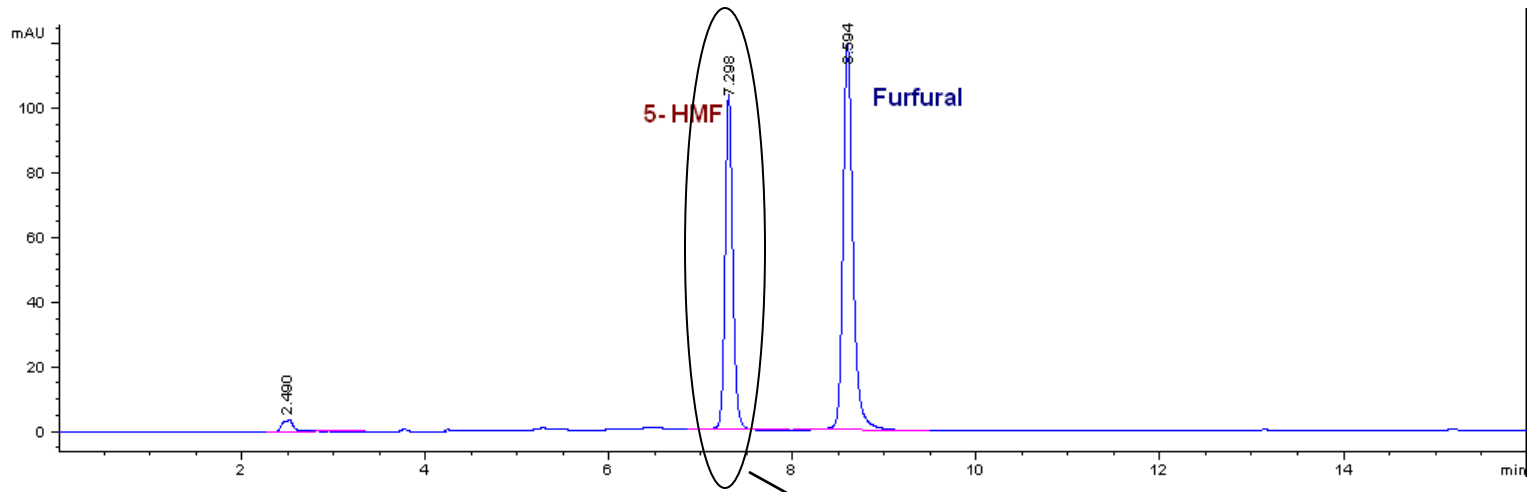
UV-Vis Spectrum of
FD&C Yellow #5

Prohibited Ingredients (Continued)

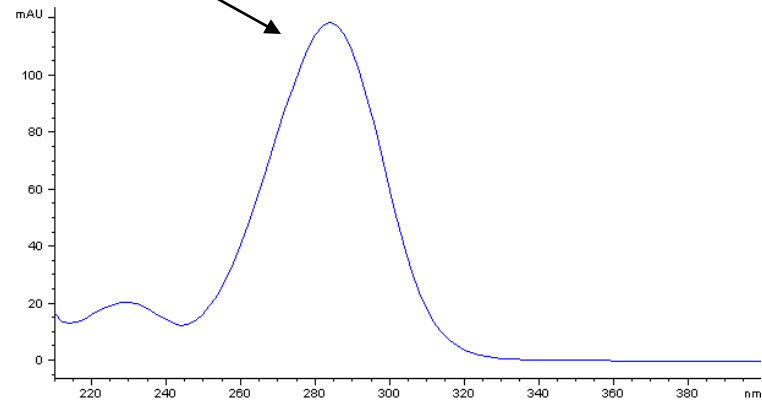
- Caramel in Straight Whisky (Standard of Identity):
 - Caramel may be added to a blended whisky to impart color
 - Synthetic caramel is typically produced by the dehydration of hexoses forming 5-hydroxymethylfurfural (5-HMF)
 - No pentoses, therefore no furfural (FF)
 - According to 27 CFR 5.23(a)(3)(iii) no material whatsoever may be added to a straight whisky
 - 5-HMF and FF formed naturally in the charring of new oak barrels
 - Developed an HPLC/DAD for the detection of 5-HMF and FF in whisky
 - Reversed-phase, C18 column, water and methanol as mobile phase, 275 nm
 - Method is linear down to 1 ppm
 - An FF/5-HMF ratio of less than 2.0 indicates the addition of caramel

Prohibited Ingredients (Continued)

HPLC Chromatogram of a Whisky Spiked with 5-HMF and FF



UV-Vis Spectrum of 5-HMF

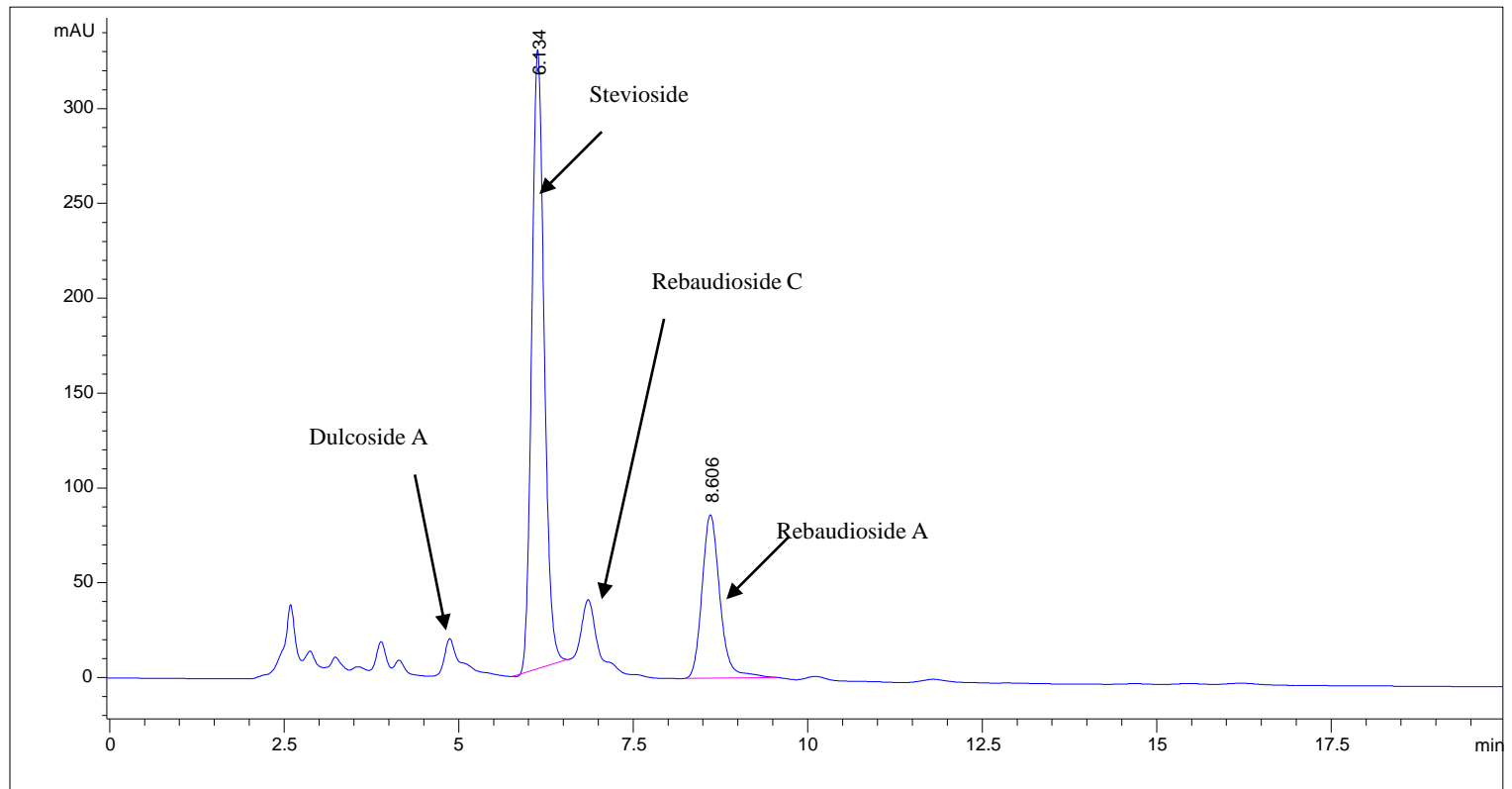


Prohibited Ingredients (Continued)

- Stevioside and Rebaudioside A:
 - Found in extract of *stevia rebaudiana*
 - Sweetener often used in alcoholic beverages such as soju
 - Stevioside is prohibited for use in food or beverages, highly purified reb A recently approved by FDA
 - Developed an HPLC/DAD method for the detection of stevioside
 - Aminopropyl column, 250 mm x 4 mm, 5 um particle size
 - Acetonitrile/water mobile phase – isocratic
 - 205 nm

Prohibited Ingredients (Continued)

HPLC Chromatogram of a Stevia Extract



Limited Ingredients

Limited Ingredients

- Typically, FDA establishes if an ingredient is limited for use in food and/or beverage:
 - Methanol
 - Sulfur dioxide
 - Flavoring substances
 - Quinine
 - Caffeine
 - Prussic acid

Limited Ingredients (Continued)

- Methanol:
 - Byproduct of fermentation
 - FDA has set a safe level of methanol at 0.1% of methanol by volume in wine; any wine containing methanol in excess of this amount is deemed adulterated pursuant to the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 342(a)(2)(C) and 348 (Industry Circular 93-3)
 - FDA has established a safe level of methanol at 0.35% v/v in fruit brandy (FDA Administrative Guides 7401.01 and 1701.01) — TTB uses this threshold for all distilled spirits

Limited Ingredients (Continued)

- Methanol:
 - Currently use a capillary GC-FID method to quantify methanol levels in alcoholic beverages
 - DB-WAXETR, 30 m x 0.53 mm, 1 um film thickness
 - Linear range of 0.01-20% v/v, LOQ=0.01%
 - Method is also used for other fusel oils, PG, ethyl acetate, acetaldehyde, acetic acid, glycerol, benzaldehyde

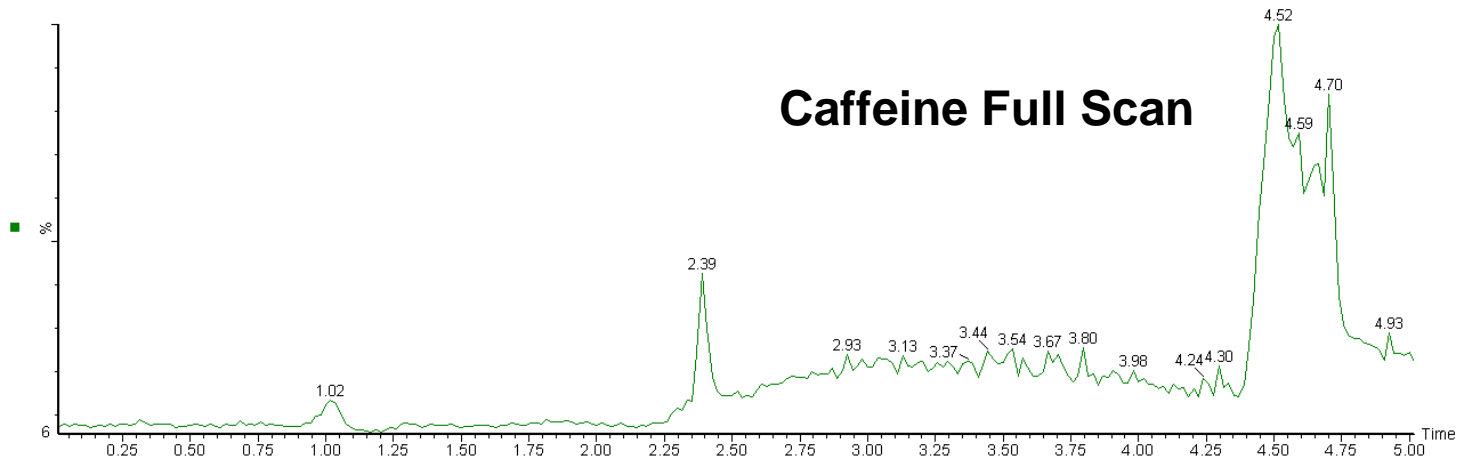
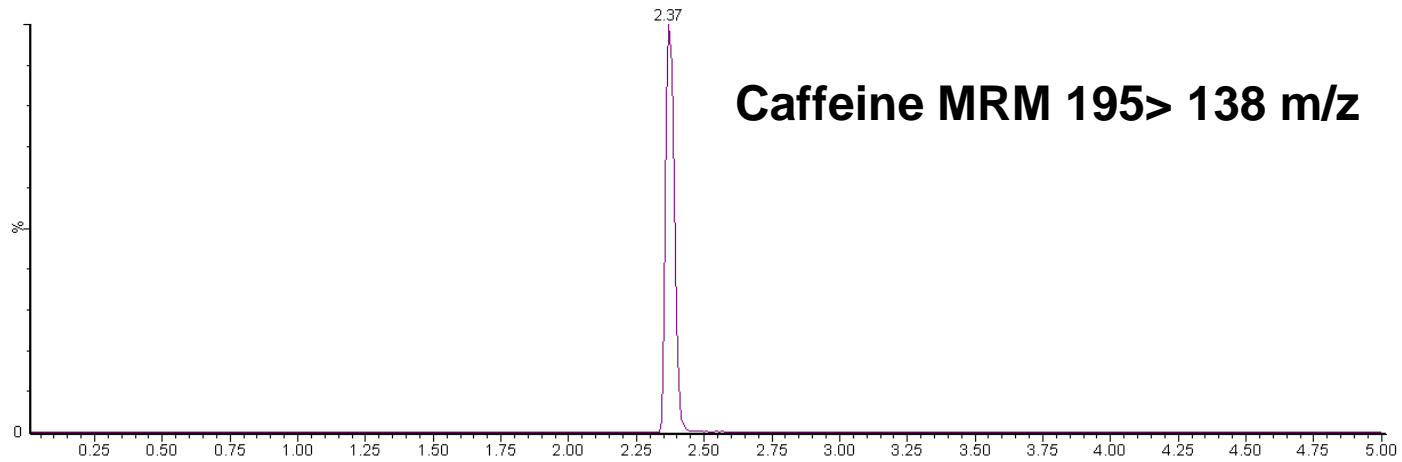
Limited Ingredients (Continued)

- Total Sulfur Dioxide:
 - Must be labeled if beverage contains 10-350 ppm (27 CFR 4.22(b)(1), 4.32(e) and 7.22(b)(6))
 - Use modified aeration-oxidation method
 - Free and bound sulfite is converted to SO₂, trapped in peroxide and converted to sulfuric acid, titrated with sodium hydroxide
 - Linear range of 5-500 ppm, LOD=1 ppm, LOQ=5 ppm
 - Currently evaluating segmented flow analyzers

Limited Ingredients (Continued)

- Quinine:
 - Limited to 83 ppm (21 CFR 172.575)
 - Use an HPLC/DAD method
 - Common marker for 5010 tax credit
- Caffeine:
 - 21 CFR 182.1180 sets a tolerance of 0.02% (200 ppm)
 - Develop a UPLC/MS/MS method
 - C18 Acquity column, +ESI mode, multiple reaction monitoring
 - Linear range of 5-500 ppm, LOD=1 ppm, LOQ=5 ppm

Limited Ingredients (Continued)



Limited Ingredients (Continued)

- Prussic acid (cyanide):
 - Found in small quantities in the following flavor substances: cherry pits, elder tree leaves, peach leaves
 - Limited to 25 ppm (21 CFR 172.510)
 - 1 ppm in wine (27 CFR 24.246)
 - Use an ion chromatography method
 - LOD = 1ppm, LOQ = 3.5ppm
 - Waters Protein PAK DEAE 5 PW
 - High dilution (100:1)

Miscellaneous

Pesticides, Trace Metals and
Ethyl Carbamate

Pesticides in Wine

- Analysis of Pesticides in Wine:
 - EPA sets regulations on the use and establishes tolerances on the residues of pesticides in foods and beverages
 - Tolerances have been established for grapes and wine grapes (cyazofamid, fluazinam, procymidone, vinclozolin)
 - No established pesticide residue levels in wine, use levels established for grapes as a guideline
 - If a pesticide is not registered with EPA, it is prohibited for use on crops (therefore prohibited for use in wine)
 - FDA and USDA monitor food sources to ensure that the limits are enforced
 - TTB has been screening wines since 1999 using a multiresidue GCMS method
 - Monitor ~155 pesticides in wine

Pesticides in Wine (Continued)

- Pesticides:
 - Agilent 6890 GC equipped w/5973A mass selective detector and automatic liquid sampler
 - HP-5ms cap column: 0.25mm, 30m, 0.25um, constant pressure mode using retention time locking
 - SIM mode using three programs
 - Organohalogen
 - Organonitrogen
 - Organophosphorus
 - QC samples
 - 50 ppb standard
 - Water blanks and spikes
 - Wine blanks and spikes

Extracted Ion Chromatogram -Dimethomorph I and II

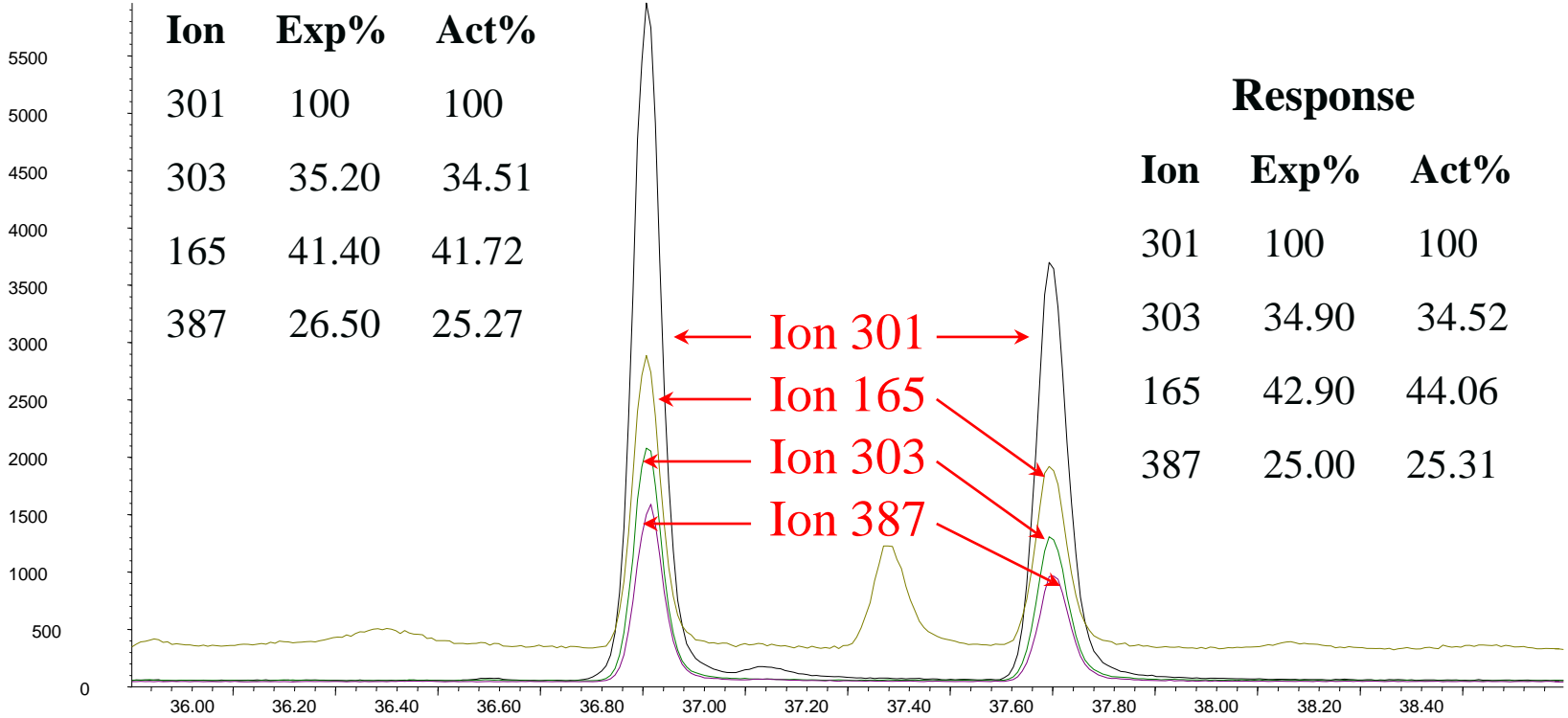
Abundance

Response

Ion	Exp%	Act%
301	100	100
303	35.20	34.51
165	41.40	41.72
387	26.50	25.27

Response

Ion	Exp%	Act%
301	100	100
303	34.90	34.52
165	42.90	44.06
387	25.00	25.31



Time-->

Trace Metal Analysis

- Typically, FDA establishes if a trace metal is limited for use in a food and/or beverage:
 - Industry Circular 91-11 sets a tolerance of 300 ppb lead in wine
 - 27 CFR 24.246 sets a tolerance of 500 ppb copper in wine
 - If fined with copper sulfate
 - Inductively Coupled Plasma Mass Spectrometry
 - Direct analysis (dilute and shoot)
 - Low ppb LOQ
 - High throughput
 - Able to scan for 53 elements in one sequence
- If TTB analyzes a sample, and the concentration of a trace metal appears to be high, we ask FDA for a health hazard evaluation

Ethyl Carbamate

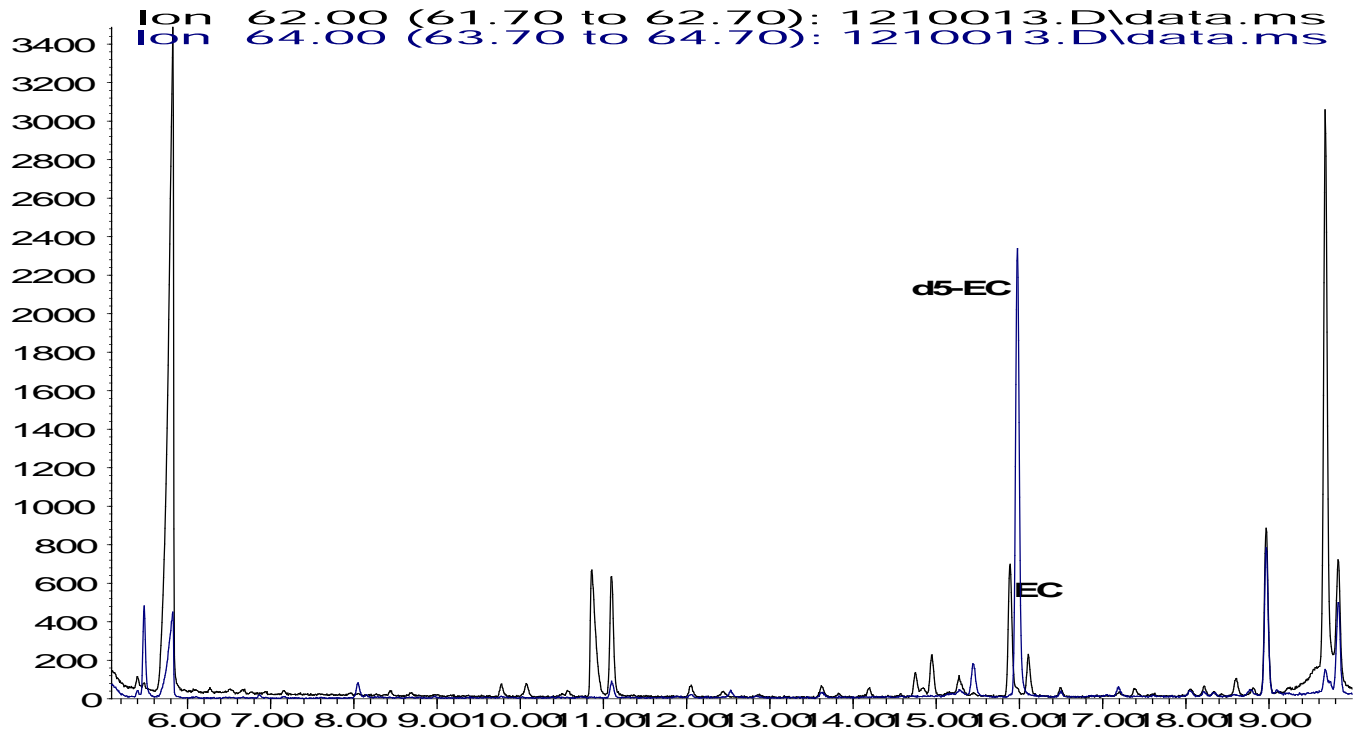
- Presence of EC in food and beverages is a public health concern for FDA and some foreign governments
 - Canada has imposed regulatory limits on EC
 - 30 ppb table wines, 100 ppb fortified wines, 150 ppb most distilled spirits, 400 ppb fruit brandies
- EC formation in wine may be due to reaction of antimicrobial agent diethyl pyrocarbonate (DEPC) with ammonia
 - DEPC is now prohibited for use in wine

Ethyl Carbamate (Continued)

- EC can also form due to the reaction of urea and ethanol in aqueous media
- AOAC method involves liquid-liquid extraction with methylene chloride
- Beverage Alcohol Laboratory has developed an optimized SPE method using GC/MS
 - Use deuterated EC as an internal standard

Ethyl Carbamate (Continued)

Abundance



Time-->

**Extracted ion chromatogram of a whisky sample
spiked with deuterated ethyl carbamate internal standard**

Conclusions

- SSD applies modern analytical techniques in the analysis of limited and prohibited ingredients
- Our method development program is modeled after AOAC OMA Program Manual

Food Allergens

Background and Regulatory Issues

Food Allergies

- Incident rate:
 - ~1.5% for adults
 - ~6-8% for children under 3
- Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) requires that food product label declare if a “major food allergen” is present, as well as ingredients that contain protein derived from major food allergens
- Plain, common language of the “BIG 8”
 - Milk, egg, fish, wheat, tree nut, peanut, soy, shellfish
- The allergen is a protein

Food Allergy/Intolerance/ Hypersensitivity

- Immunological:
 - Antibody mediated (food allergy)
 - Cell mediated (celiac disease)
- Non-immunological:
 - Metabolic (lactose intolerance)
 - Toxicological (scombroid poisoning)

TTB and FALCPA

- FALCPA does not directly apply to alcoholic beverage labels
- The House Committee on Energy and Commerce called for TTB to work with FDA on appropriate labeling
- Clear intent in the House Committee Report is that TTB issue regulations similar to FALCPA standards

Food Allergens and Regulations

- On April 29, 2005, TTB published an ANPRM titled “Labeling and Advertising of Wines, Distilled Spirits and Malt Beverages; Request for Public Comment”
 - Broad announcement to public
- Invited comments on labeling, calorie and carbohydrate claims, serving facts, ingredient labeling and allergen labeling
- Consumers, interest groups, trade associations, industry, other members of the public
- Received 18,000 comments, 50 of which concerned allergens

Comments from ANPRM

- Industry cost versus consumer benefit
- Requirement of a full list of ingredients in addition to the allergen label
- Separate labeling when an allergen appears as part of a brand name
- Labeling of processing and fining agents
- Setting of thresholds
- Harmonization with foreign government requirements

Comments from ANPRM (Continued)

- Comments on Labeling When the Allergen Appears as Part of Brand Name:
 - “Creek’s Wheat Beer”
 - “Wheat Creek Lager”
 - Is the brand name imparting information about the presence of an allergen?
 - Best policy to have one standard format, regardless of the brand name

Comments from ANPRM (Continued)

- Labeling of processing and fining agents:
 - FDA defines a processing aid as an “incidental additive” and must conform to FALCPA
 - “Processed with” versus “contains”
 - “May contain” is unclear and misleading for a processing aid that was intentionally added

Comments from ANPRM (Continued)

- Harmonization with Foreign Government Requirements:
 - On Nov 25, 2003, the European Commission implemented a provisional exclusion for 8 major uses of food allergens in alcoholic beverages (end Nov 27, 2007)
 - Distillates made from cereal with gluten, whey and nuts
 - Lysozyme, albumin, fish gelatin (isinglass) and casein products used in wine
 - Nuts used as flavor in distilled spirits

Food Allergens and Regulations

- Of the comments TTB received on allergens, vast majority favored mandatory labeling
- As a result, TTB has published both an NPRM and an interim rule involving a period of voluntary labeling of food allergens
- On July 26, 2006, TTB published an NPRM soliciting comments regarding the adoption of mandatory food allergen labeling for wines, distilled spirits and beer
 - Comment period extended to Dec 26, 2006
 - Focused announcement to public

Food Allergens and Regulations (Continued)

- TTB published interim rule T.D. TTB-53, 71 FR 42260, which sets forth standards for optional allergen labeling statements
 - Any reference made to allergens must declare all major food allergens used in the production of the beverage, including fining or processing agents
 - An allergen declaration must consist of the word “Contains” followed by a colon and the name of the food source from which each major food allergen is derived. For example, a declaration could be “Contains: milk and egg.”

Food Allergen Labeling – Exemptions

- Highly refined oil
- Exemption under the FD&C Act:
 - Petition to exempt an ingredient (180 days; FDA has rejected one; one petition pending)
 - Notification to exempt an ingredient (90 days; FDA has objected to all seven notifications submitted)
- Exemption from TTB regulations

Petition Process (if Labeling is Mandatory)

- Potentially can petition TTB to exempt an alcoholic beverage from food allergen labeling if demonstrate:
 - Product does not cause an allergic response that poses risk to health
 - Burden is on the petitioner to provide scientific evidence
 - Product does not contain an allergenic protein, even though a major food allergen was used — Burden is on the petitioner to provide scientific evidence
- TTB will focus on products not ingredients
- Lab must develop methods to verify the presence or absence of food allergens in alcoholic beverages

Food Allergens

Detection and Evaluation

Analytical Methods to Detect Food Allergens

- Polymerase Chain Reaction:
 - Expensive
 - Need specific primers
 - Specific
- Mass Spectrometry:
 - Expensive
 - Not high throughput, technically demanding
 - Specific
- Enzyme Linked Immunosorbant Assay:
 - Inexpensive (\$300-\$500/kit, up to 96 wells)
 - Amenable to high throughput

Fining Agents Used for Wine

- Used to achieve clarity, improve color and flavor:
 - Bentonite (clay), 2-8 lbs/1,000 gal
 - Agar (polysaccharides)
 - Synthetic polymers (PVPP), 1-6 lbs/1,000 gal
 - Kieselsol (silicon dioxide), <10 lbs/1,000 gal
 - Carbon, 3 lbs/1,000 gal
 - Protein (milk, gelatin, fish, egg)

Protein Fining Agents for Wine

- Milk:
 - Principle protein is casein, soluble at high pH
 - Usually use potassium caseinate (or cellulose adduct – VINPUR)
 - White wine clarifier, 0.2-2 lbs/1,000 gal
- Gelatin:
 - Protein in connective tissue
 - Soften red wines, 0.2-0.8 lb/1,000 gal
- Isinglass:
 - Fish protein, 0.2-0.5 lb/1,000 gal
- Egg:
 - Egg whites, 3 egg whites/60 gal

Egg Allergens

- Egg whites contain 9-11% protein, most allergenic proteins found in egg white:
 - Ovomucoid (11% of egg white protein)
 - Ovalbumin (54%)
 - Ovotransferrin (12%)
 - Lysozyme (3.5%)
- Allergenic potential for egg yolk is only moderate
- Evaluate commercially available ELISA kits for egg protein in wine

ELISA Technology

- Enzyme-linked immunosorbent assay
- Assay proteins
- Utilizes antibodies (monoclonal or polyclonal) immobilized on polystyrene — Capture antibody
- Second antibody is labeled with an enzyme (sandwich type) — Detector antibody
- Enzyme reacts with substrate, then add chromagen followed by stopping reagent
- Color detected by plate reader; intensity is quantitated

Egg Protein Assay ELISA Kits

- Neogen-Veratox[®] Quant Egg Allergen Test
 - Wine validation method
- Biopharm-Ridascreen[®] Ei/Egg Protein
 - Antibodies specific to egg white protein
- Tepnel-Egg Assay Kit
 - Polyclonal antibody to ovomucoid
- Morinaga Institute Egg Protein ELISA Kit
 - Specific to ovalbumin

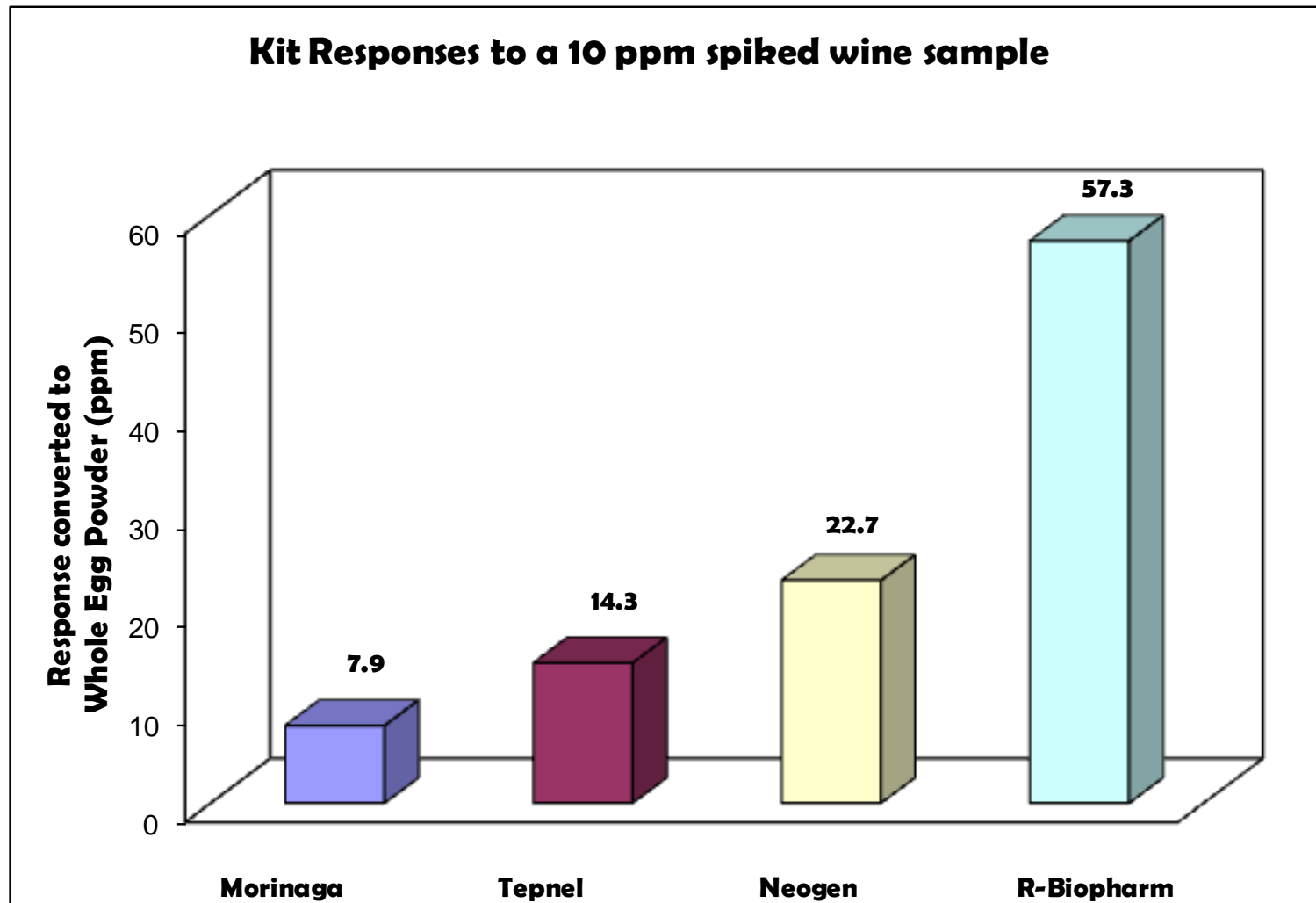
Egg Protein Assay ELISA Kits (Continued)

- Each kit supplied with STD, but each kit STD is different
 - Tailored for maximum binding to kit antibody
 - Antibodies are usually proprietary
 - Used a commercially available spray-dried whole egg powder (reference material obtained from S. Hefle, 48.9% total protein)
- Must dilute concentrated samples to fall in range of calibration curve
 - Each time run is performed, need calibration curve of kit standards and run in duplicate

Wine Samples

- Obtained commercially available wine samples directly from the manufacturer through our Trade Investigation Division
- Nine red wine samples:
 - Cabernet, Pinot, Merlot, Zinfandel, Syrah
 - All fined with egg except for Syrah, used as control and spiked with reference material
- One white wine sample:
 - Chardonnay
 - Not fined with egg, used as control and spiked with reference material

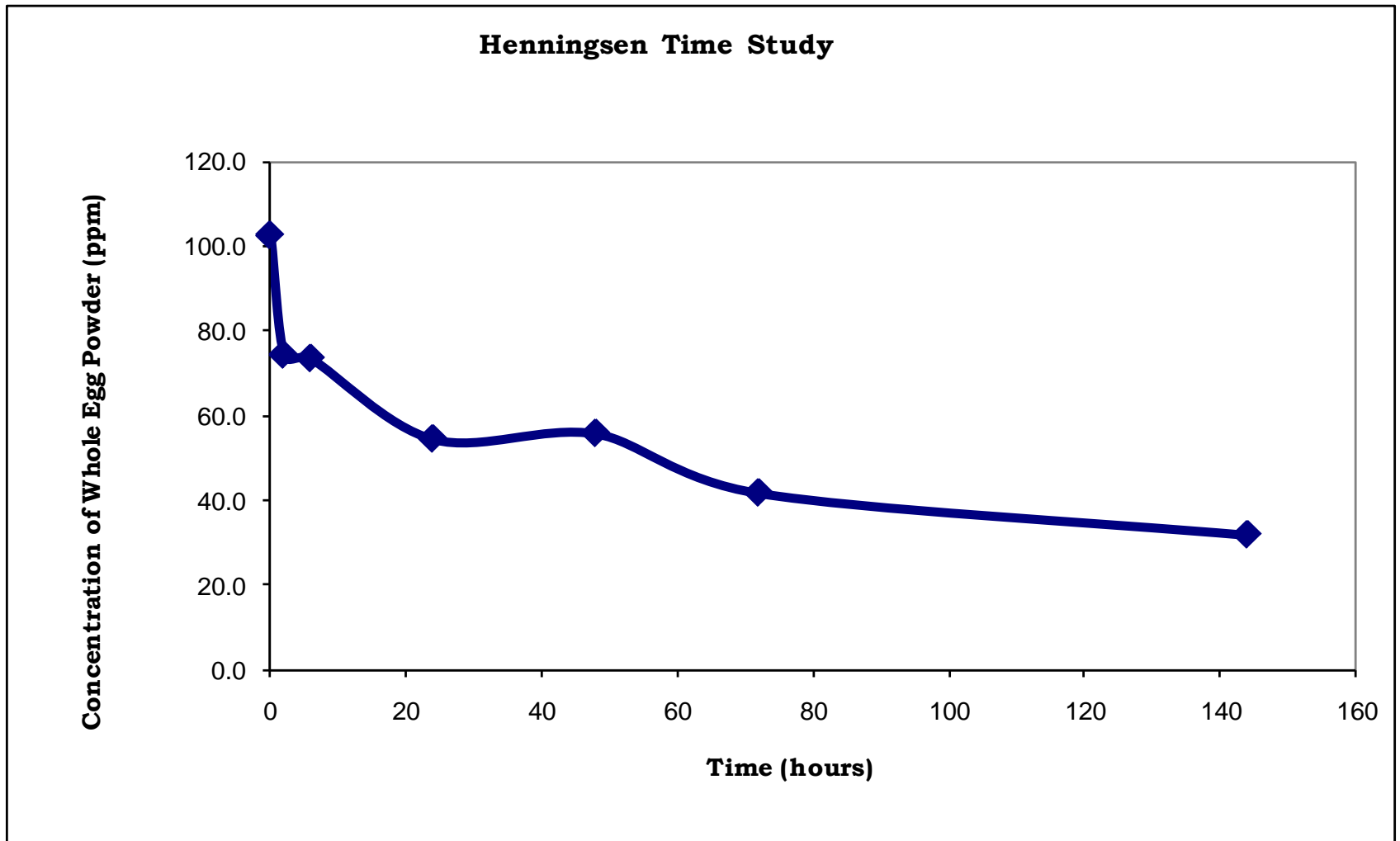
Egg Kit Responses to Wine Spiked with RM



Wine Fined with Eggs

- All kits detected egg protein in spiked wine matrices
- None of the kits detected egg protein in fined samples
- Limit of Quantitation:
 - Neogen, 2.5 ppm, (1 ppm with wine method)
 - R-Biopharm, 2 ppm
 - Tepnel, 0.5 ppm

Response of Egg RM vs. Time



Conclusions on Egg Kits

- ELISA technology evaluated
- Egg reference material spiked in wine matrices was detected by all kits; however, wide disparity in responses
- Response to egg reference material shown to degrade over time in wine
- Results from ELISA test will not be sufficient for allergen label claim exemptions

Wheat Allergens

- Gluten is group of cereal proteins comprised of 50% glutelins and 50% prolamines – prolamines are soluble in 40-70% aqueous ethanol
- Cereals are used as source for malt beverages – barley
- Other cereal adjuncts may be used in brewing process – corn, oats and wheat

Gluten ELISA Kits

- Gliadin, a wheat prolamine, involved with allergies (antibody response) and intolerances (cell-mediated response)
- Is this protein in malt beverages?
- Evaluate commercially available ELISA-based kits for gluten detection in malt beverages
- FDA has authority to issue guidelines to establish a definition of “gluten-free”

Analyzed Malt Beverage Samples

- 3 Domestic
- 2 Domestic light
- 3 Import
- 2 Import light
- 1 Stout
- 1 Lager
- 1 Ale
- 1 Nonalcoholic
- 2 Flavored malt beverage
- 2 Wheat beer

Gluten Assay ELISA Kits

- Neogen-Veratox[®] Quant Gliadin Test:
 - Proprietary
- R-Biopharm-Ridascreen[®] Fast Gliadin:
 - R5 Antibody (produced by Mendez in Spain)
 - Peptides containing the QQPFP sequence are recognized by R5 antibody
 - R-Biopharm now has a competitive ELISA
 - Recognizes gliadin epitope; however, results are in peptide equivalents, not in ppm gluten

Gluten Assay ELISA Kits (Continued)

- Tepnel- Gluten Assay Kit:
 - Skerritt Antibody
- Each kit supplied with STD, but each kit STD is different:
 - Tailored for maximum binding to kit antibody
 - Variables include: ionic strength, carrier proteins, enhanced solubility

Other Gluten ELISA Kit Issues

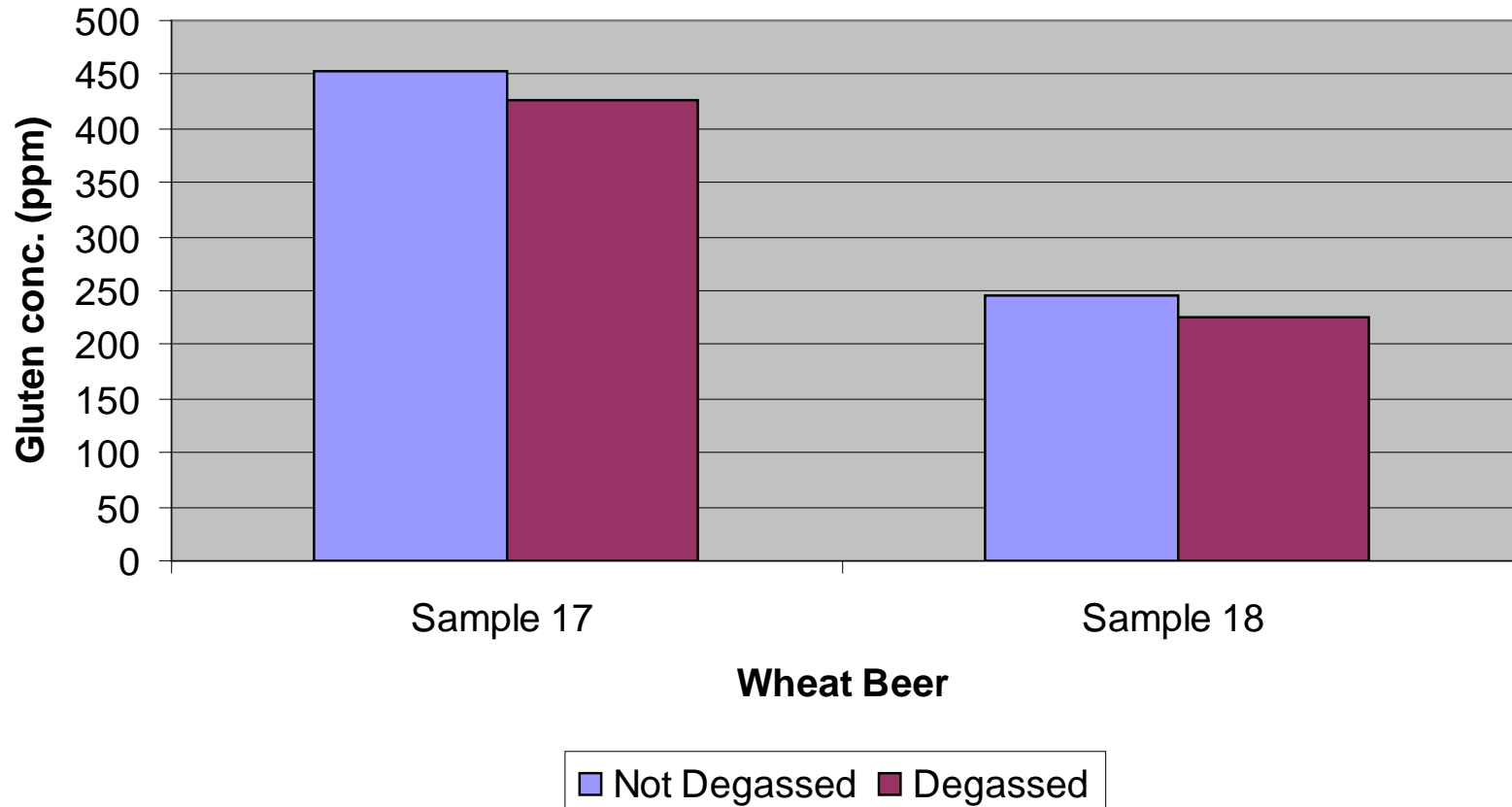
- Expiration date
- Must be stored at 4° C
- Specificity:
 - Kits only detect gliadin, celiac patients may also react to glutelins
- What to use as reference material?
- Limit of Quantitation:
 - 5-10 ppm for all kits evaluated

Sample Preparation

- Extract malt beverage in 40-70% EtOH solution usually 10:1
- Dilute even further in sample diluent for a total of 400-500:1 dilution
- Sample preparation issues:
 - Degassing
 - Extracting in ethanol
 - Minimizing non-specific binding
 - Tannin binding solution
 - Heat treated samples
 - Pre-extraction cocktail

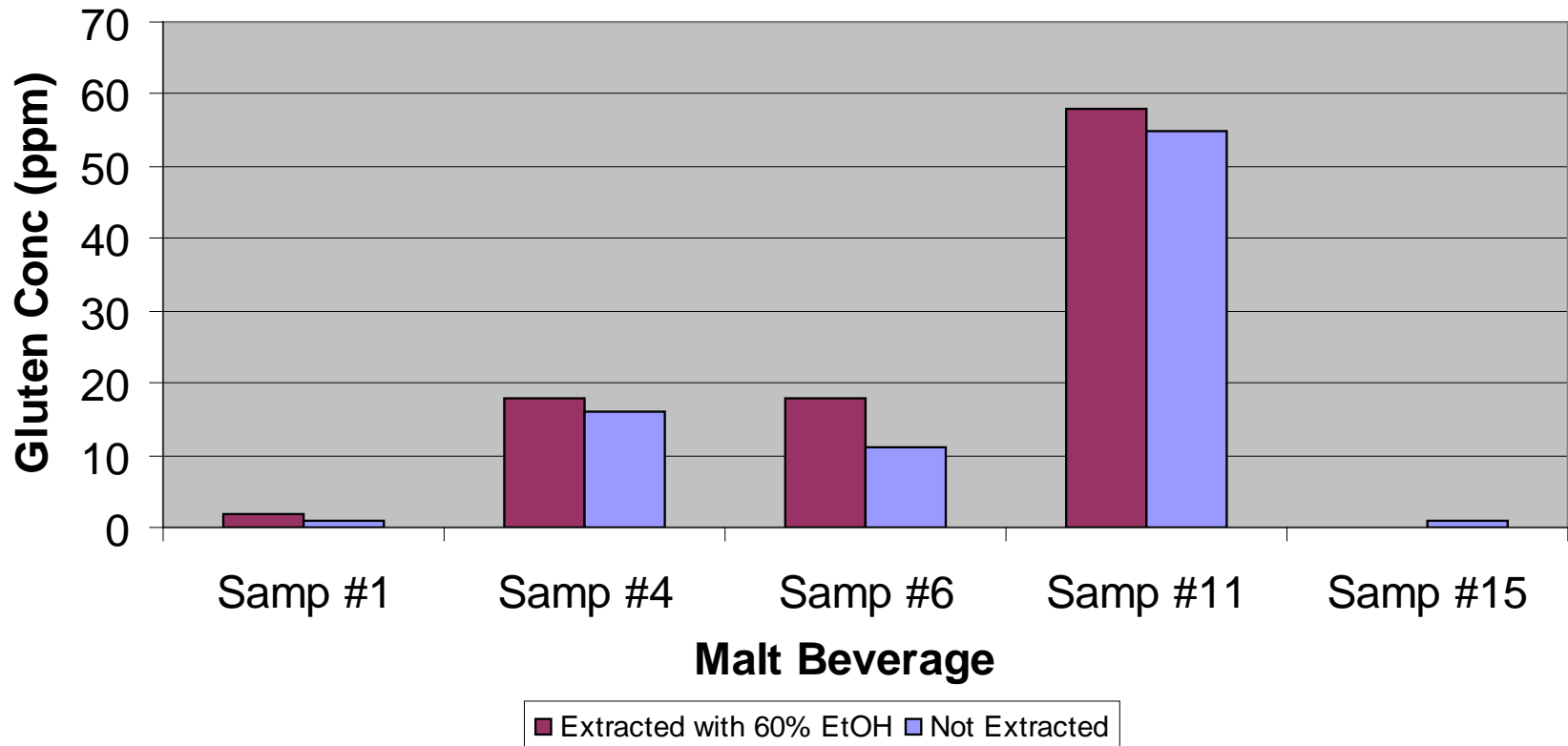
Results

R-Biopharm Degassing Experiment

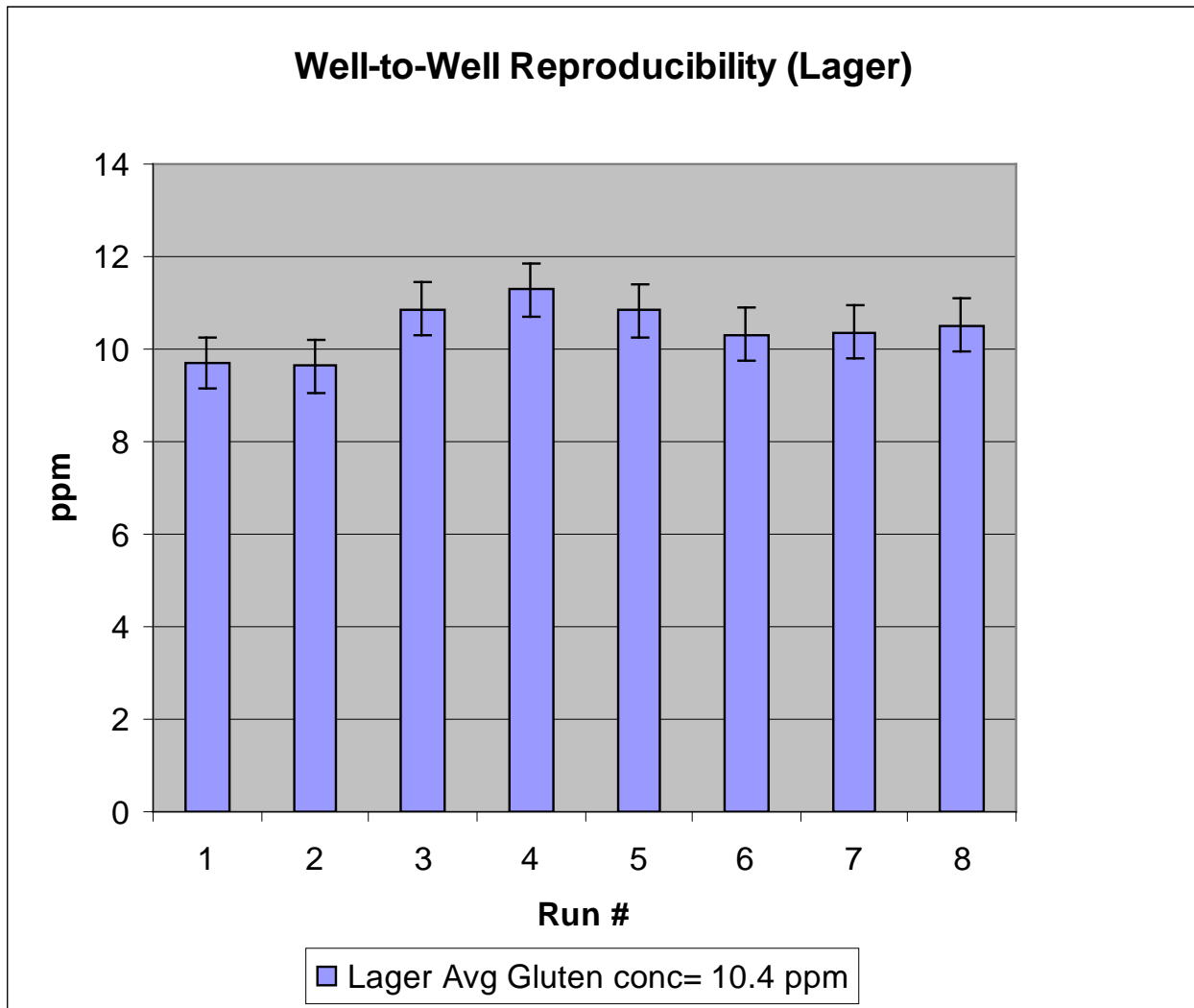


Results (Continued)

R-Biopharm Ethanol_(aq) Extraction Experiment

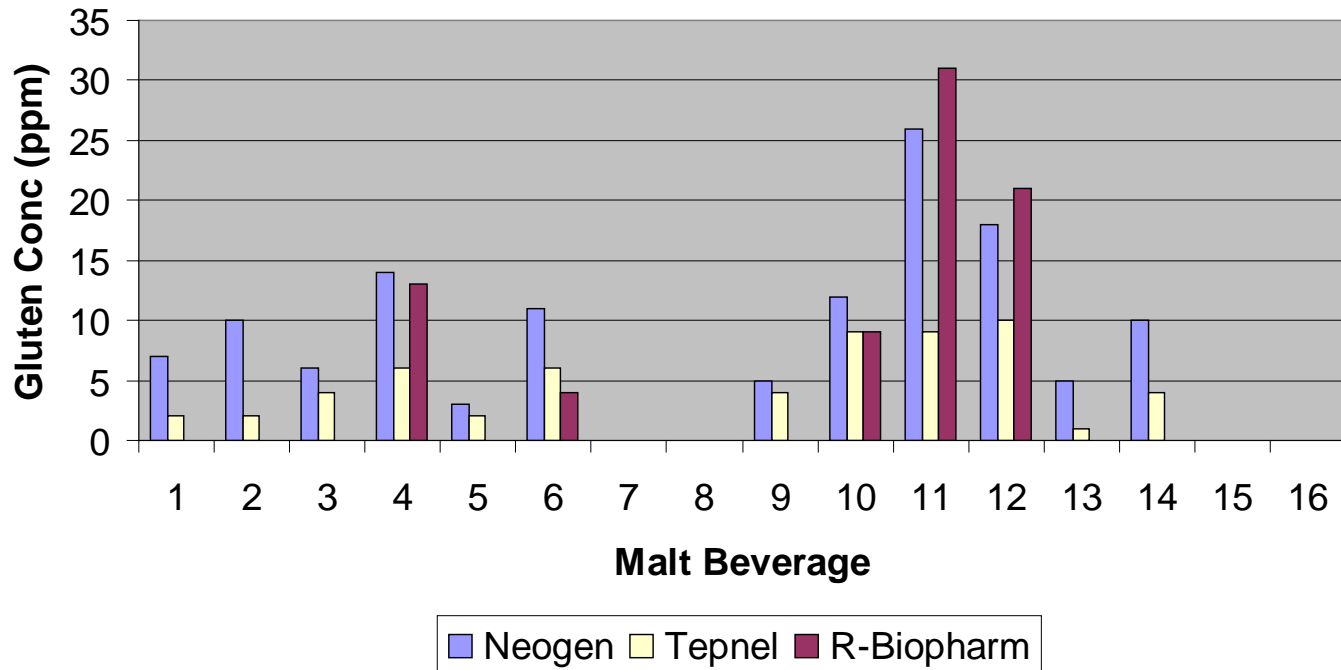


Results (Continued)

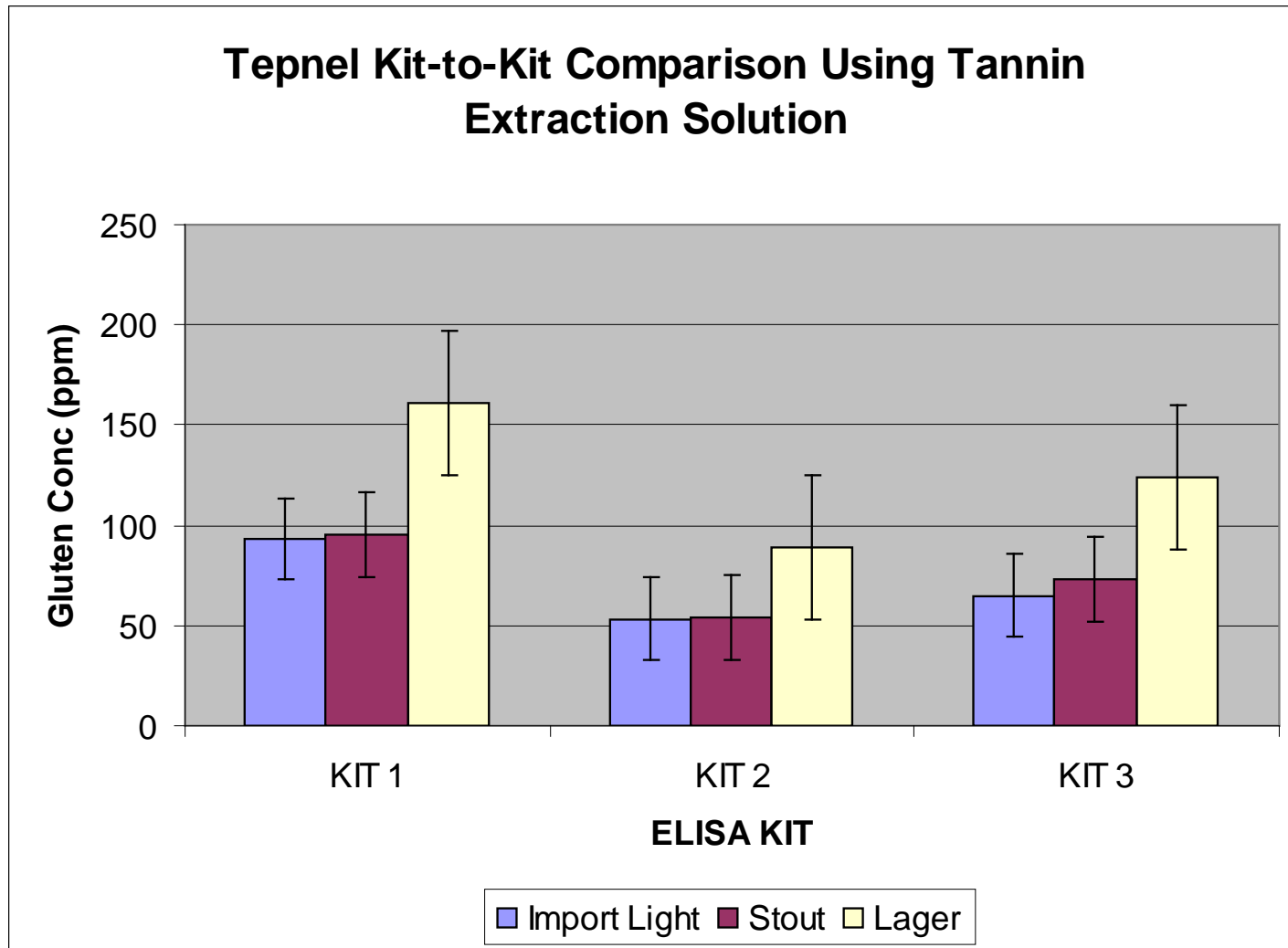


Results (Continued)

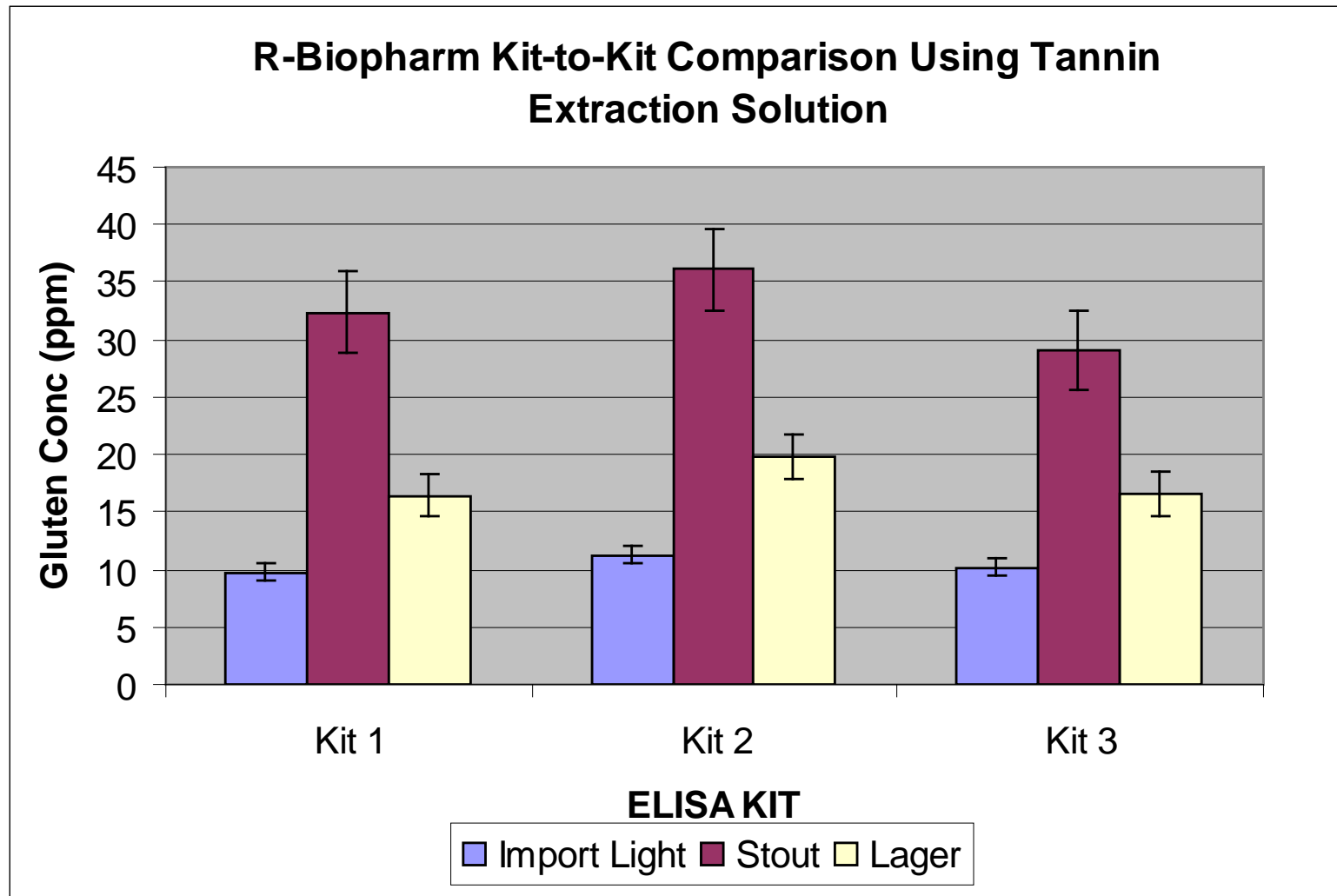
Comparison of Gluten Conc in Malt Beverages by Various Kits (no Tannin Extraction Soln)



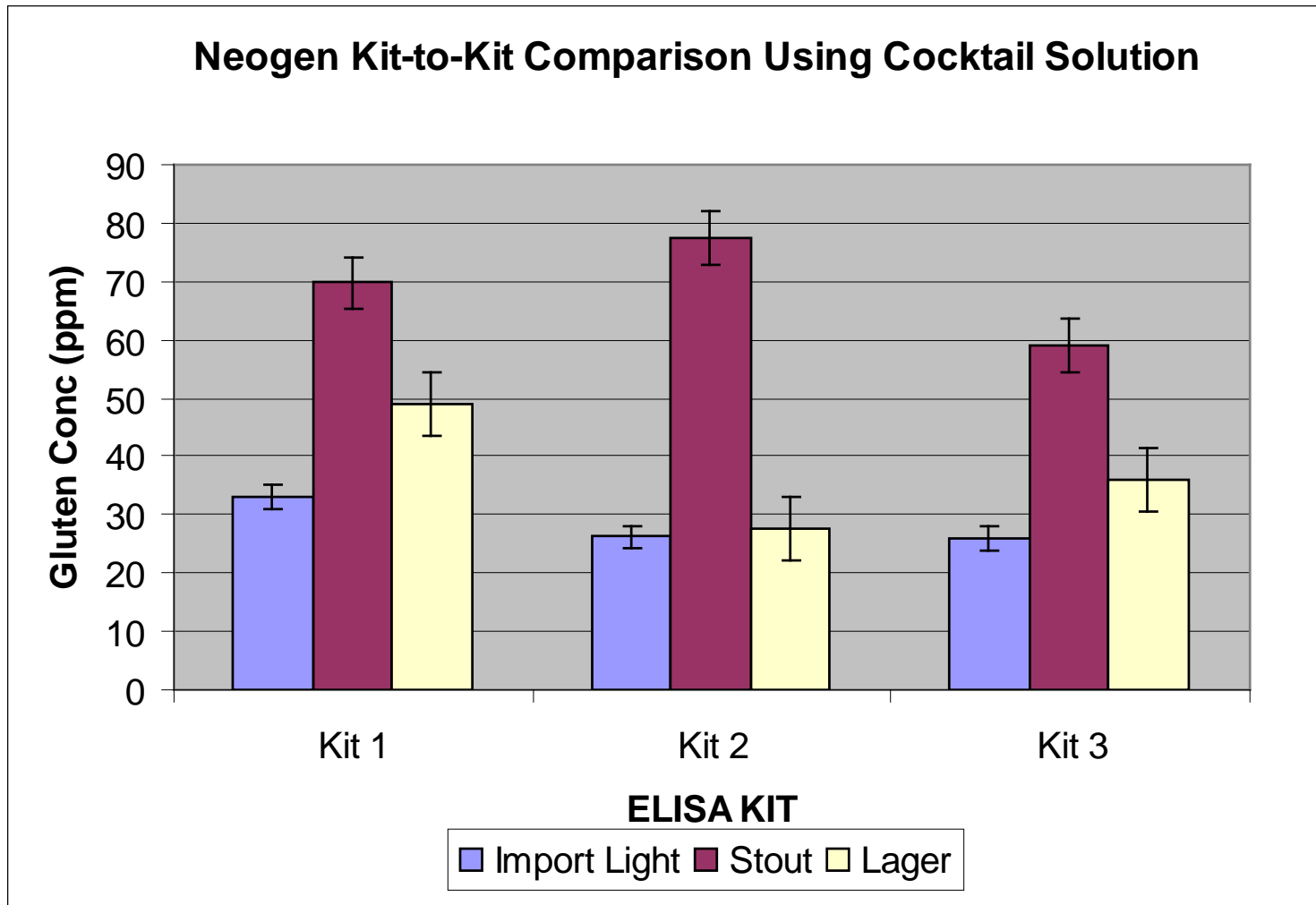
Results (Continued)



Results (Continued)

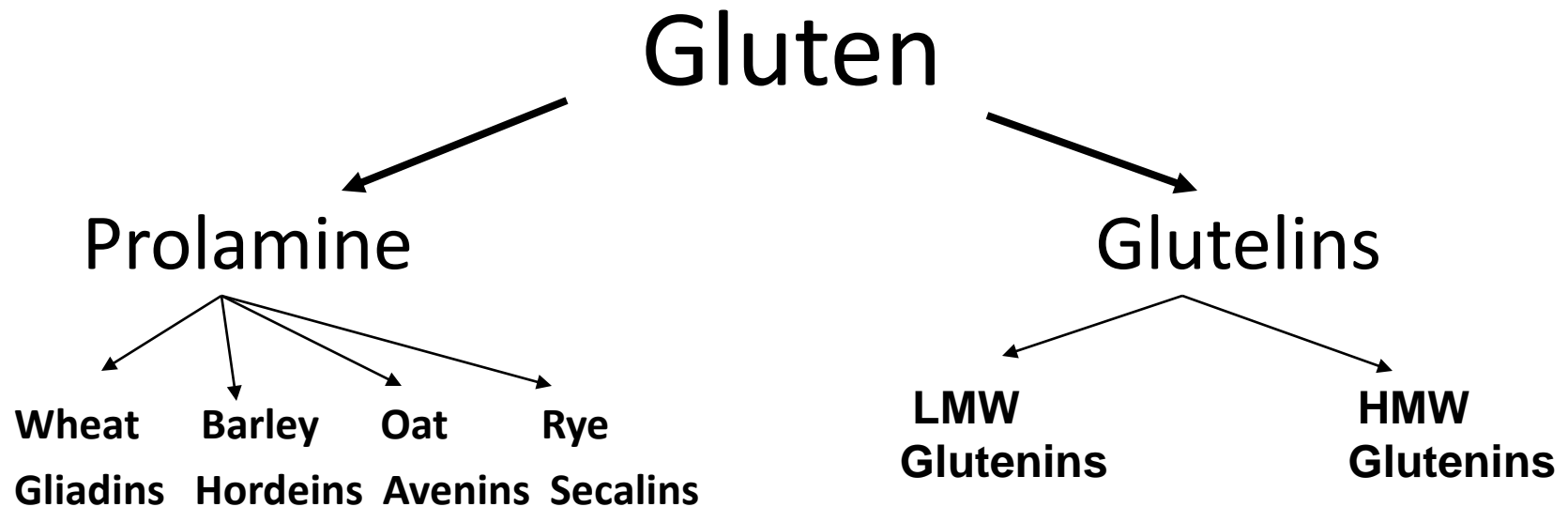


Results (Continued)



Non-ELISA Detection of Food Allergens

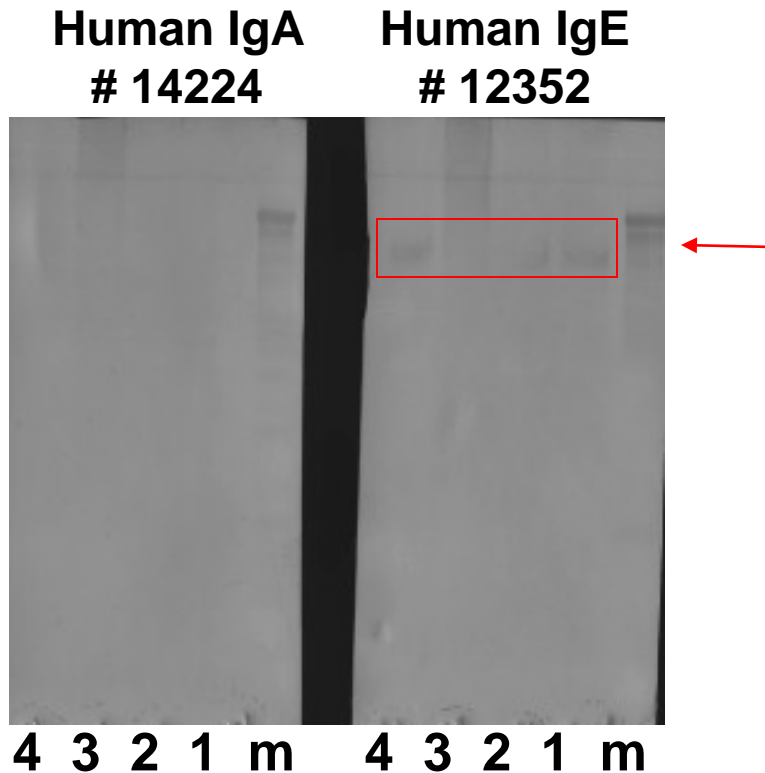
Gluten



- Mol Wt 30-50kDa
- Rich in proline and glutamine (20%,35%)
- Monomeric polypeptide chain

- Mol Wt 15-150kDa
- Many intra- and intermolecular disulfide bonding due to high cysteine residues
- 13% proline, 30% glutamine

Evaluation of Malt Beverage Protein Profiles via Immunoblot



- Evaluated 4 beers: 1) wheat, 2) lager, 3) stout, 4) light; (m=marker)
- Membrane evaluated for IgA, no binding
- Membrane evaluated for IgE, only high MW binding (glutelins)
- Will require very sensitive techniques

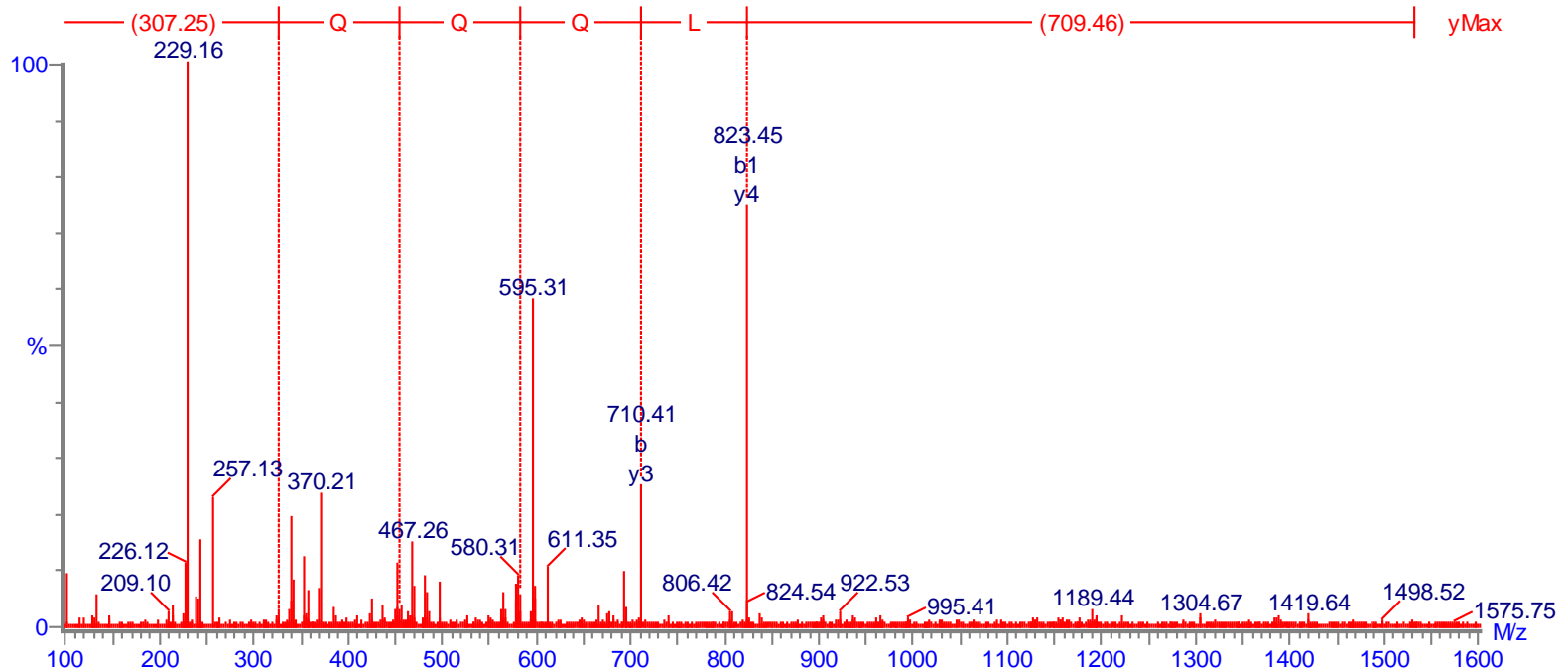
Protein Detection by Mass Spectrometry

- May detect hydrolyzed or denatured proteins
- Very selective in determining the grain origin of proteins
- Quantification capabilities with triple quadrupole detectors
- Protein Sequencing and Identification
 - Protein Purification
 - Proteolytic Digest (Trypsin)
 - Mass Spectrometric Analysis of Tryptic Digest
 - Protein Identification by Database Searching

50ug/ml Gluten digest 30% EtOH

kjs_Gluten_041107_2 MaxEnt 3 37 [Ev61619,lt50,En1] (0.050,200.00,0.200,1400.00,2,Cmp)

9: TOF MSMS 766.96ES+



MSMS spectrum of the fragments of 766.96 m/z ion extracted from one of the gluten digest peak eluting at 40.89 min. The dotted lines represent sequences and peptide masses obtained using the peptide sequence tag analysis.

Conclusions

- TTB mandated by Congress to enforce FALCPA
- Mandatory food allergen labeling is proposed but not yet implemented
- Petitions for label exemptions possible
- ELISA technology evaluated
- All kits detected gluten in malt beverages, quantitation difficult
- Results from ELISA test will not be sufficient for allergen label claim exemptions
- TTB is evaluating the use of mass spectrometry to identify and quantitate allergens in alcoholic beverages

Web Sites

www.ttb.gov

<http://www.cfsan.fda.gov>

<http://ecfr.gpoaccess.gov>

<http://www.farrp.org/index.htm>

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