Paradigm Shift: The Importance of Closing the Characterization Gap Between APIs & Formulation Components

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Overview

Introduction to High Purity Carbohydrates

The Shifting Paradigm (Focusing on Characterization)

Quantitative Analysis Beyond ICH/Pharmacopeia Requirements

Case Studies

Q&A

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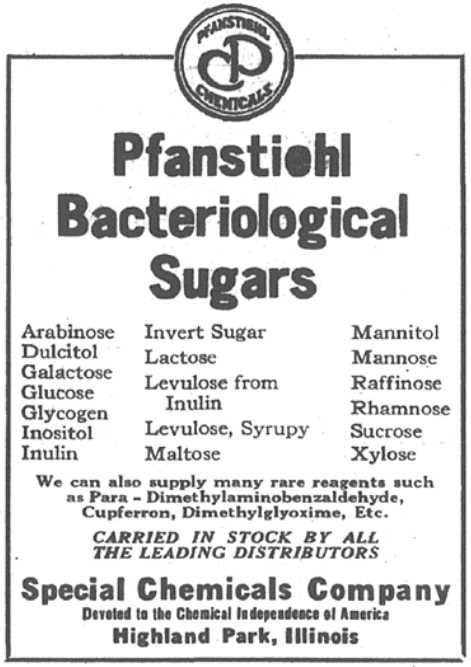
Pfanstiehl - A Market Leader in Biopharma Components

US-based cGMP Manufacturer of High Purity Low Endotoxin Components for Biologics, Vaccines, Cell Culture Media & other Injectables (liquid & lyo)

- Trehalose
  - Sucrose
    - Galactose
      - Mannose
        - Mannitol
          - Maltose



The Scientist, 1920

"Tried & True" Parenteral Excipients & APIs for 50+ Years

Utilized in the majority of Top 10 Biotech Blockbusters

Market leader in FDA-approved trehalose-containing biologics

Market leader in FDA-approved sucrose-containing biologics

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Introduction to High Purity Carbohydrates

Primarily Mono & Disaccharides

Manufactured Under cGMP

99.0 - 99.9% Pure

Endotoxin Levels 0.05 – 2.5 EU/g

Suitable for Parenteral Applications

Multi-Compendial

ICH Compliant

Treated More and More as APIs

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High Purity Carbohydrate Applications

Stabilization of Proteins, Cells, Particles

Modulation of Cell Metabolism to Improve Product Quality

Lyo/Cryoprotection

Yield Enhancement

APIs

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Trehalose: Approved Therapeutics

Table 1: Some Approved Drugs Formulated with Trehalose

Drug	Manufacturer	API Class	Formulation Type
Adcetris	Seattle Genetics	ADC	Lyophilized Powder
Avastin	Genentech/Roche	mAb	Solution
Blincyto	Amgen	Bispecific mAb	Lyophilized Powder
Cosentyx	Novartis	mAb	Solution
Gazyva	Genentech/Roche	mAb	Solution
Herceptin	Genentech/Roche	mAb	Lyophilized Powder
Lucentis	Genentech/Roche	mAb	Solution
Dengvaxia	Sanofi	Vaccine	Lyophilized Powder
Adynovate	Baxalta/Shire	rProtein	Lyophilized Powder
Tanzeum	GSK	rProtein	Subcutaneous
MabThera	Genentech/Roche	mAb	Subcutaneous

Sampling of Marketed mAbs & ADCs Using Trehalose & Sucrose

Manufacturer	Drug	Form	Drug Class	Excipient	*Excipient Amt.	
					(mg/mL)	(%)
Seattle Genetics	Adcetris	Lyophilized	ADC	Trehalose	70	7%
Genentech/Roche	Avastin	Solution	mAb	Trehalose	60	6%
	Herceptin	Lyophilized		Trehalose	18.2	2%
	Lucentis	Solution		Trehalose	100	10%
	Gazyva	Solution		Trehalose	90.8	9.08%
Amgen	Enbrel	Solution	Fusion Protein	Sucrose	10	1%
	Enbrel	Lyophilized		Sucrose	10	1%
	Blincyto	Lyophilized	Bis mAb	Trehalose	34	3%

***Often times, the highest usage is during purification, rather than in the final formulation, in order to improve yield, retain native conformation.**

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Trends in Biopharma Components/Excipients

- Increased Regulatory Scrutiny
  - **cGMP required**
  - Multicompendial criticality, updates to mongraphs, including ChP
  - **Excipients increasingly treated and/or utilized as APIs**
  - Enhanced Change Control Requirements
- Enhanced Purity & Characterization
  - **Elemental Impurities (ICH Q3D)**
  - Impurity Profiling
  - Glucans vs Endotoxins
  - **Specific Component Impacts on Stability (e.g. iron, copper, zinc, manganese)**
- Moving beyond the pharmacopeia (next gen components)
  - **Safety & Quality vs Functionality**
  - Process Robustness/Reproducibility
  - Customized Excipients
  - Systems/Platform Approaches
- Stabilizers Being Utilized Farther Upstream (Cell Culture & Purification)
- Trehalose as a Preferred Material

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“Old Paradigm” vs “Emerging Paradigm)

Old Paradigm

- Formulation Components Are Inactive Ingredients w/Limited Functionality
- The Specification Tells Us All We Need To Know
- “Pass”, “Meets”, “NMT”, “>”, “<“ are Good Enough
- Minor Changes in Impurity Profiles Are Unlikely to Impact Stability
- “Sucrose is Sucrose”

Emerging Paradigm

- Formulation Components Can and Do Play a Significant Role in Therapeutic Stability, Quality, Potency, Immunogenicity, and Bioavailability
- Quantitative Characterization Beyond ICH/MOnograph is Necessary
- This Data is Needed to Make Safety & Functionality Decisions/Correlations
- We Don’t Just Need to Know Something is Present, We Also Need to Know What It Is and How it Varies, How We Control It, Similar to APIs

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Embracing the New Paradigm

Current Test Name	Description of Change/Comments
Color and Clarity of Solution	Tighten specification for compliance with ChP
Color and Clarity of Solution	Tighten specification for compliance with ChP
Identification (HPLC)	Add ID by HPLC retention time for compliance with ChP
Heavy Metals (as Pb)	Replaced with Elemental Impurities method
Endotoxins	Tighten specification based on historic performance
Assay	Change to quantitative impurities method
Total Impurities with RRT <1.0	Change to quantitative impurities method
Total Impurities with RRT >1.0	Change to quantitative impurities method
Impurity A (Glucose)	Change to quantitative impurities method Update name to be consistent with Impurity B
Impurity B	Change to quantitative impurities method Update name to specify this limit is for Impurity B Tighten specification based on historic performance
Any Other Impurities	Change to quantitative impurities method
Total Impurities	Change to quantitative impurities method
Residual Ethanol	Tighten specification based on historic performance
Residual Methanol	Tighten specification based on historic performance
Total Aerobic Microbial Count	Tighten specification based on historic performance
Total Combined Molds and Yeasts Count	Tighten specification based on historic performance
Staphylococcus aureus	Add to provide comprehensive microbial evaluation of product
Pseudomonas aeruginosa	Add to provide comprehensive microbial evaluation of product
Cadmium	Add for compliance with ICH Q3D
Lead	Add for compliance with ICH Q3D
Arsenic (inorganic)	Add for compliance with ICH Q3D
Mercury (inorganic)	Add for compliance with ICH Q3D
Nickel	Add for compliance with ICH Q3D
Molybdenum	Add for compliance with ICH Q3D
Copper	Add for compliance with ICH Q3D
Chromium	Add for compliance with ICH Q3D
Iron	Add for compliance with ICH Q3D
Aluminum	Add for compliance with ICH Q3D
Zinc	Add for compliance with ICH Q3D

Building a Database for Our Clients:

- **Everything on the COA**
- **Sub-Visible and Nano-Particulates**
- **Glucans**
- **Data for Other On-Market Materials Our Clients May be Using (competitor materials)**
- **Custom Testing as Needed**
- **Safety & Functional Ramifications/Correlations Drive Control and Testing Strategies**

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Analytical Results (T-104-4, Trehalose)

Batch Consistency (32 Batches)											
Test	Assay	RRT 0.85 (%)	Imp B (%)	Glucose (%)	Total Impurities (%) 1	Conductivity (µS/cm)	Karl Fisher	Residual Ethanol (ppm)	Residual Methanol (ppm)	TAMC	TYMC
Ave	99.8	0.001	0.13	0.01	0.12	1.5	9.6	51	5	<10	<10
STD	0.57	0.001	0.03	0.00	0.03	0.2	0.2	16	2		

Elemental Impurities (ppb) (24 Batches)											
Element	Cd	Pb	As	Hg	Ni	Mo	Cu	Cr	Al	Fe	Zn
Ave	0	0	0	1	2	0	2	14			
STD	0	0	0	1	3	0	1	3			

Subvisible Particulates (Average # per particulate size) (5 Batches)												
Particulate Size	0.2 - 0.5 µm	0.5 - 0.75 µm	0.75 - 1 µm	1 - 1.5 µm	1.5 - 2 µm	2 – 5 µm	5 - 10 µm	10 - 15 µm	15 - 20 µm	20 - 25 µm	25 - 50 µm	Ave Total / g
Ave	2	23	63				12	1	0	0	0	

Endotoxin / Glucans		
Test	Glucans (ng/g)	Endotoxins (EU/g)
# Batches Tested	10	31
Ave	One batch at 0.2 all others at <0.155*	
* Result < LOQ of method utilized		

(1) Impurity Levels determined by proprietary HPLC methods internally developed for the purposes of batch to batch analysis.

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Analytical Results (S-124-1-MC, Cane Sucrose)

Batch Consistency											
Test	Assay (%)	Raffinose (area %)	Glucose (area %)	Fructose (area %)	RRT 1.7 (area %)	Total Impurities (area %)	Conductivity (µS/cm)	Residual Methanol (ppm)	Residual Ethanol (ppm)	TAMC	TYMC
# Batches Reviewed	16	16	16	16	16	16	32	33	33	14	14
Ave	99.7	0.01	0.02	0.01	0	0.04	1	9	223	<1	<1
SD	0.4	0.00	0.02	0.01	0	0.03	0.4	4	83	<1	<1

Elemental Impurities (ppb) (23 Batches)											
Element	Cd	Pb	As	Hg	Ni	Mo	Cu	Cr	Al	Fe	Zn
Ave	0	0	1	0	3	0	14	12			
STD	0	0	0	0	4	0	6	2			

Subvisible Particulates (Average # per particulate size) (5 Batches)												
Particulate Size	0.2 - 0.5 µm	0.5 - 0.75 µm	0.75 - 1 µm	1 - 1.5 µm	1.5 - 2 µm	2 - 5 µm	5 - 10 µm	10 - 15 µm	15 - 20 µm	20 - 25 µm	25 - 50 µm	Total
Ave	2	20	47				13	2	2	1	1	

Glucans / Endotoxin / 5-HMF / Furfural (Average)				
Test	Glucans (pg/mg)	Endotoxins (EU/g) Glucans blocked	5-HMF (ppb)	Furfural (ppb)
# Batches Reviewed	20	37	10	10
Ave		<0.05	25	1
* Result < LOQ of method utilized				

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Analytical Results (S-124-2-MC, Beet Sucrose)

Batch Consistency											
Test	Assay (%)	Raffinose (area %)	Glucose (area %)	Fructose (area %)	RRT 1.7 (area %)	Total Impurities (area %)	Conductivity (µS/cm)	Residual Methanol (ppm)	Residual Ethanol (ppm)	TAMC	TYMC
# Batches Reviewed	10	10	10	10	10	10	10	25	25	25	25
Ave	99.0	0.030	0.009	0.001	0.005	0.006	0	13	256	<10	<10
SD	0.2	0.010	0.001	0.003	0.002	0.003	0	4	74		

Elemental Impurities (ppb) (20 Batches)											
Element	Cd	Pb	As	Hg	Ni	Mo	Cu	Cr	Al	Fe	Zn
Ave	0	0	0	0	1	0	7	11			
STD	0	0	0	0	1	1	6	4			

Subvisible Particulates (Average # per particulate size) (5 Batches)												
Particulate Size	0.2 - 0.5 µm	0.5 - 0.75 µm	0.75 - 1 µm	1 - 1.5 µm	1.5 - 2 µm	2 - 5 µm	5 - 10 µm	10 - 15 µm	15 - 20 µm	20 - 25 µm	25 - 50 µm	Total
Ave	0	15	42				10	1	0	0	0	
SD	1	9	8				6	1	1	0	1	

Glucans / Endotoxin / 5-HMF / Furfural (Average)				
Test	Glucans (pg/mg)	Endotoxins (EU/g)	5-HMF (ppb)	Furfural (ppb)
# Batches Reviewed	23	10	10	10
Ave		<0.05	18	0
SD			33	0
* Result < LOQ of method utilized				

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Nano-particulate Analysis (Sucrose & Trehalose)

Product	D10 (nm)	D50 (nm)	D90 (nm)	Nanoparticulate Counts - Conc (part/g)
	10 Lots Sample for Each Code			
S-124-1 (Sucrose - Cane)				
Ave	67	100	172	
SD				
Min				
Max				
S-124-2 (Sucrose – Beet)				
Ave	70	100	163	
SD				
Min				
Max				
Measurement: Acquired from PTL Laboratories utilizing a Malvern NanoSight Instrument				

Product	D10 (nm)	D50 (nm)	D90 (nm)	Nanoparticulate Counts - Conc (part/g)
	11 Lots Sampled			
T-104-4 (Trehalose)				
Ave	101	149	222	
SD				
Min				
Max				
Measurement: Acquired from PTL Laboratories utilizing a Malvern NanoSight Instrument				

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Case Study #1 – mAb Product Quality

- Recently Approved Monoclonal Antibody
- Client Experienced a Sudden Shift in Glycosylation Profile
- No Immediate Obvious Root Cause
- Initiated Investigation of >60 Cell Culture Media Components
- Quickly Resolved by Trending the Elemental Profile of Historic Galactose Batches and Identifying a Correlating Shift in Metal Profile
- The Levels of Metals Found to Impact the Product Quality Were Well Below Those Required by ICH Q3D (low ppb levels)
- The Shift in Metal Content Would Not Have Been Detected, Let Alone Quantified, Under The “Old Paradigm”
- Control Strategy Had Already Been Implemented by Pfanstiehl
- Client Has Now Expanded Use Testing and Supplier Requirements as a Result

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Case Study #2 – Glucan vs Endotoxin

- Client Manufacturing FDA-Approved Monoclonal Antibody
- Having Issues with Elevated Endotoxin Levels During Downstream Processing
- Engaged Pfanstiehl to Determine if it Could be Originating from the Sucrose Used During Purification to Prevent Aggregation
- Quantitative Analysis of Endotoxin Showed No Correlations to Pfanstiehl Lots
- However, Contribution of Glucan to Endotoxin Levels was Unknown
- Pfanstiehl Developed a Quantitative Glucan-Specific Method and Generated a Batch History to Provide Transparency to the Contribution of Each Sucrose Lot
- Client Was Able to Determine that Their Process Was Actually Concentrating the Glucans and Giving an Artificially High Endotoxin Test Result
- Better Understanding of This Issue Allowed Both Parties to Put in Place Additional Control Strategies to Ensure the Issues Would Not Recur

Challenges for Next Generation Components

ICH compliance according to API standards

Emerging Applications Requiring Enhanced Characterization

- Highly Concentrated Formulations (i.e. subcutaneous)
- Opthalmic & Inhalation delivery
- Cell Therapy & Gene Therapy
- Increasingly complex & insoluble therapeutics (i.e. ADCs)
- Novel Drying Technologies (i.e. shelf-stable vaccines)

Increased Understanding of Trace Component Impacts Will Drive:

- Need for improved consistency, process robustness
- Tightening of specifications, quantitative reporting (i.e. particulates, metals, reducing sugars, unknown impurities, glucans)
- Novel excipients & stabilization “systems”

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Conclusions

- **Quantitative Characterization Beyond ICH or a Monograph is Critical**
- Strong Engagement Between Suppliers and Formulation Scientists is Needed to Correlate Very Detailed Chemical Characteristics with **Functional Peformance**
- cGMP Manufacturers of Excipients Should Be Taking a Leadership Role in **Closing the Gap between API & Excipient Standards**
- **Collaboration** between **Excipient Manufacturers & Formulators** needed to define “Next Generation” Requirements.