A Quality-by-Design Approach Used to Develop an Accurate and Precise Enzyme-Linked Immunosorbent Assay (ELISA) for the Determination of Milk Allergens in Hypo-Allergenic Powdered Infant Formula

October 19, 2017

Introduction

Product Goal

Nutritious Caseine-based powdered-infant formula (IF) that will be safe for infants with confirmed milk allergy

Background

- Casein (CAS) accounts for approximately 80% of protein in cow's milk
- CAS (allergen) is immunoactive
- Hydrolysis breaks CAS into smaller-sized peptides
- Extensively-hydrolyzed (EH) Casein Raw Material (RM)
- Potential sources of Casein in IF:
    1. Residual intact Casein from hydrolysis in the RM
    2. Contamination during IF manufacture

Critical Quality Attribute: Hypoallergenicity

Critical Quality Measure: Casein concentration in RM, IF

Process – Analytical Method

Project Goal

Analytical method (AM) capable of detecting CAS in RM and IF finished product (FP)

Analytical Method Requirements

- Accuracy
- Precision
- Sensitivity

Enzyme-Linked Immunosorbant Assay (ELISA) meets these requirements

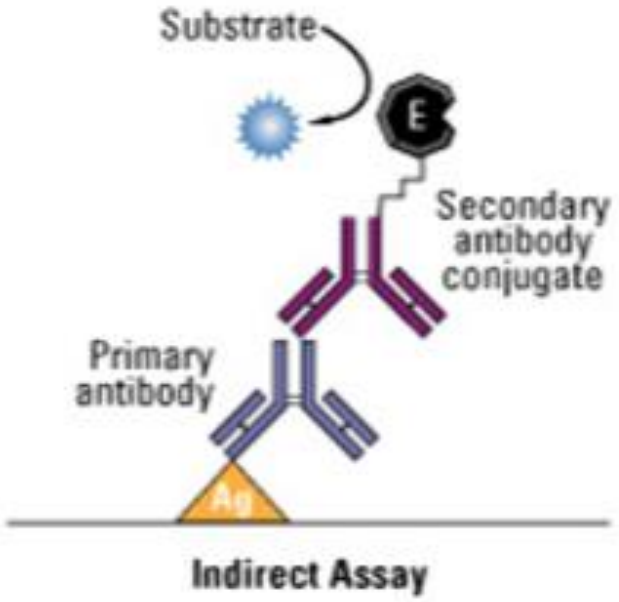
Process Description – ELISA (Assay)

- Sample Diluted
- Sonicated
- Shaken
- Centrifuged

- Bind CAS to Plate
- Bind 1º Ab to CAS
- Bind 2^0 Ab-HRP to 1º Ab

- TMB (Chromogenic Substrate) (Blue) →
- Stop (Yellow)
- UV Absorbance @ 450 nm
- Signal proportional to Casein concentration

Process Description – Indirect ELISA



Process Output (Response) – Recovery

Validation

Demonstrate that AM is suitable for intended application

Control Sample

FP sample incurred with 20 ppm casein

Purpose

To evaluate performance characteristics of the AM

Recovery

Fraction of theoretical casein concentration in a sample measured

$$\text{Recovery} = \frac{\text{Measured Casein Concentration (ppm)}}{\text{Theoretical Casein Concentration (ppm)}} \times 100\%$$

Target Recovery = 100%

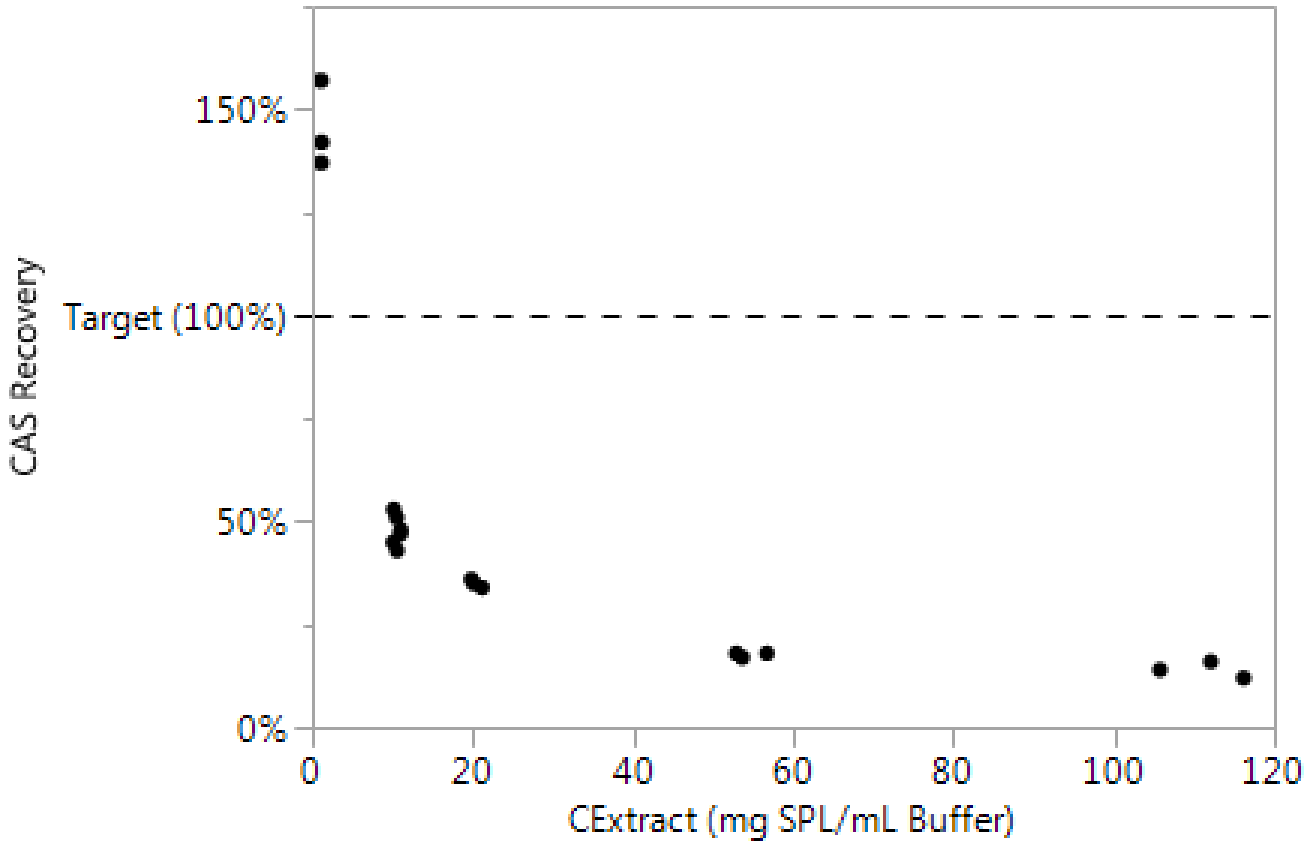
Process Inputs (Factors)

- Extraction Conc. ($C_{Extract}$)
- Extraction Buffer Composition
- Sonication Time
- Shake Time
- Centrifugation Time

- Reagent volumes
- Incubation times

- λ_{UV} = 450 nm

Predicting $C_{Extract}$ for 100% Recovery



Predicting $C_{Extract}$ for 100% Recovery

- ✓ Extraction Conc. ($C_{Extract}$)
- Extraction Buffer Composition
- Sonication Time
- Shake Time
- Centrifugation Time

- Reagent volumes
- Incubation times

- ✓ UV 450 nm

Mixture DOE for Extraction Buffer Robustness

Extraction Buffer

- 6-Component System
- Inputs – Component Volume Fractions
- Used Development Extraction Buffer Composition as Starting Point:

Component	Volume Fraction
Tris, 1M	0.025
NaCl, 2.5M	0.06
SDS, 10%	0.01
SDC, 10%	0.1
NP-40, 10%	0.1
Water	0.705

DOE

- Classical Mixture Design
- 21 Runs
- Each input varied ± 10%

Mixture DOE for Extraction Buffer Composition

- ✓ Extraction Conc. ($C_{Extract}$)
- ✓ Extraction Buffer Composition
- Sonication Time
- Shake Time
- Centrifugation Time

- Reagent volumes
- Incubation times

- ✓ UV 450 nm

DOE for Physical Extraction Parameters

Extraction Buffer – Inputs at Starting Set-Points

Physical Extraction Parameters

Parameter (Unit)	Low Value	Start Set-Points	High Value
$C_{Extract}$ (mg/mL)	2	2.5	3
Sonication (min.)	5	10	15
Shake (min.)	10	30	35
Centrifuge (min.)	10	20	25

DOE

- Custom Design
- 16 Runs

DOE for Physical Extraction Parameters

- ✓ Extraction Conc. ($C_{Extract}$)
- ✓ Extraction Buffer Composition
- ✓ Sonication Time
- ✓ Shake Time
- ✓ Centrifugation Time

- Reagent volumes
- Incubation times

- ✓ UV 450 nm

Pre-validation Summary

- Accuracy: 94-105% recovery from 4 to 20 ppm
- Intermediate Precision: mean = 107%, CV = 7% (24 CS, 8 ELISAs, 2 Analysts)
- Analytical Range: 4 to 40 ppm
- Functional Sensitivity: 1.3 ppm

Analytical Requirements Met!

I'd like to Thank the Following People …

- Pedro Prieto
- Cindy Barber
- Steve Tennyson
- Mike Miklus
- Rob Lievense
- Tanya Davis

… and You!