Complex Product Characterization – Most useful Techniques

Rationale for sampling, and batch-to-bath variability for various types of complex products

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Liposomes Characterisation:

Name of the test	Most useful Technique
Liposome Size Distribution	Zeta Sizer
Morphology- lamellarity, Bilayer thickness	Cryo-TEM
Lipid bilayer transition	Nano-DSC
Physical state of active substance inside the liposomes	SAXS
Free and encapsulated drug	Size exclusion followed by HPLC
In-Vitro Drug Release	SEC, Ultra centrifugation
Internal pH	Ultra filtration
Drug Related Impurities	LC, LCMS
Lipid Related impurities	LC-RI, ELSD, CAD

Characterisation of long acting Injectable (Polymer Based)

Name of the test for Polymer Characterization	Most useful Technique	Product Characterization	Most Useful technique
Polymer composition(L:G)	1H-NMR	Size Distribution	LD / SEM
Polymer molecular weight	GPC-MALLS/GPC-RI	Drug Distribution	Raman
Degree of Branching	GPC-MALLS/RI/ Viscosity	Porosity	Hg porosity meter
Block Length	13C NMR	Trace components	NMR/LC-MS
Residual monomers	GC/NMR	Drug Release	USP IV, Bottle rotating Test Tube/Water bath
Metal catalysts	ICP-OES/MS		
End Group chemistry	NMR / Potentiometry		
Glass transition temperature	DSC		

Characterisation of Peptides

Name of the test	Most useful Technique	Most Useful technique
Primary structure	N-Terminal sequencer	LC-HRMS
Secondary structure	Circular Dichroism	FT-IR / Raman
Tertiary /higher order structures	SEC-UV SEC/A4F-MALLS	SV-AUC NMR
Impurities characterisation	HPLC/UPLC HILIC / Ion-Exchange	LC-HRMS
Isoelectric point	Capillary Electrophoresis	
Bio-Assay	Cell based Assays	

Characterisation of Iron Nano Particles

Name of the test	Most useful Technique	Most Useful technique
Particle Size Distribution	Zeta Sizer	Cryo-TEM
Molecular weight Distribution	GPC	MALLS
Low molecular weight Complexes	Dialysis / Ultra filtration	ICP-MS
Labile Iron	Iron Chelation Assay	Bleomycin Assay
Surface Charge	Zeta Potential	
Iron Core Size and Morphology	AFM	TEM
Crystallinity	XRD, Synchrotron	Raman, TEM-SEAD, XANES
Iron Environment	Mossbauer spectroscopy	ESR/EPR, Raman, UV-Vis
Redox Potential	Polarography	Cerimetric titration
Magnetic Properties	VSM	SQUID
Carbohydrate Shell	FT-IR / NMR	SEC

Characterisation of Ophthalmic suspensions

Name of the test	Most useful Technique	Most Useful technique
Size Distribution	Laser Diffraction	Flow -CAM
Morphology	SEM	Morphology G3
Crystallinity	XRD	Raman
Rheology	Rheometer	Viscometer
Surface tension	Tensiometer	
Drop Size and Drop assay	LC-Assay - HPLC	
IVRT	Flow Through Apparatus	Bottle rotating Apparatus
Polymer Equivalence	SEC -MALLS	

Rationale for sampling, and batch-to-bath variability for various types of complex products

- Method variability (standard deviation)
- Product (batch to batch) variability
- No. of lots needed to represent reference product variability
- Establishment of equivalence criteria
- Setting specification

Note : Acceptance criteria depends on totality of the analytical data and not simply on the observed range of product attributes of the reference product. This is because some product attributes act in combination to affect a product's safety, purity, and potency profile; therefore, their potential interaction should be considered when conducting the comparative analytical assessment and setting specifications.

Statistical approaches for comparative assessment

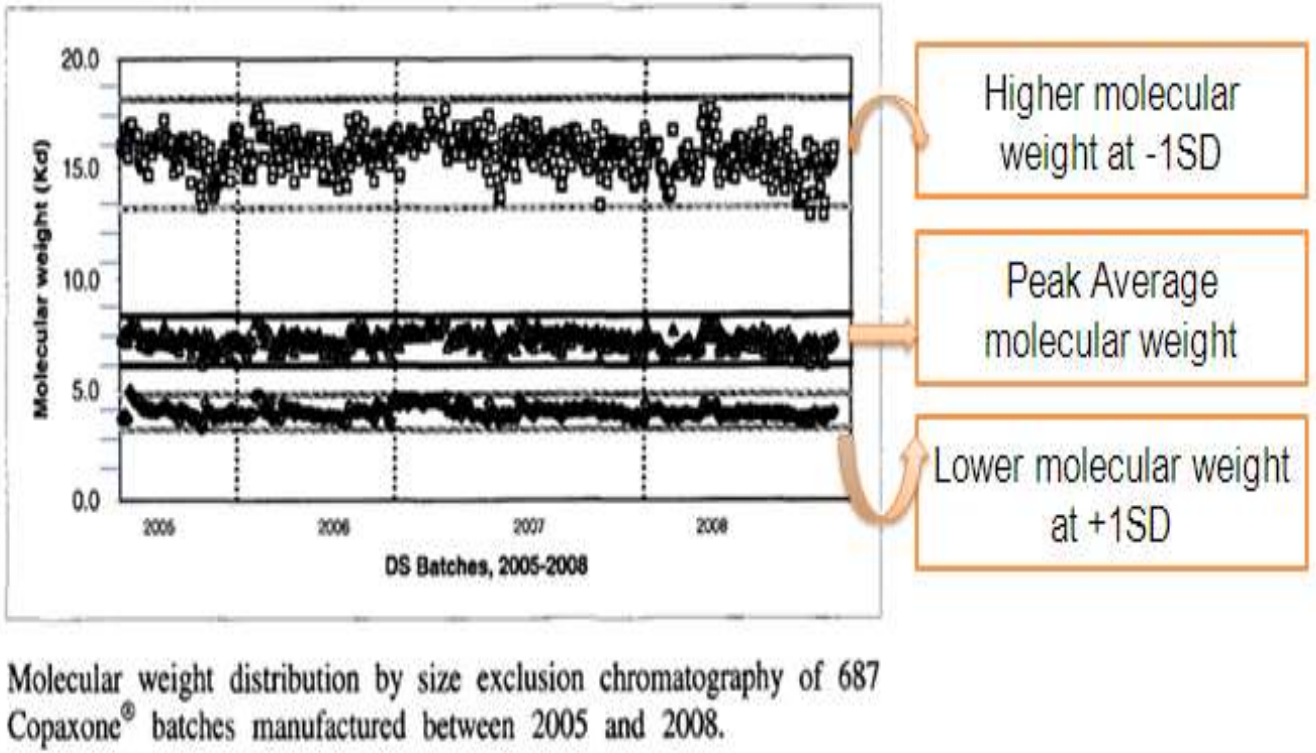
- Min-Max range along with analytical method variability
- X-Sigma: 3**σ** **(coverage 99.7%)**
- Tolerance Interval (coverage : 95 to 99% and confidence 95%)

Inferential statistical methods: Allowing for a statistical quantification.

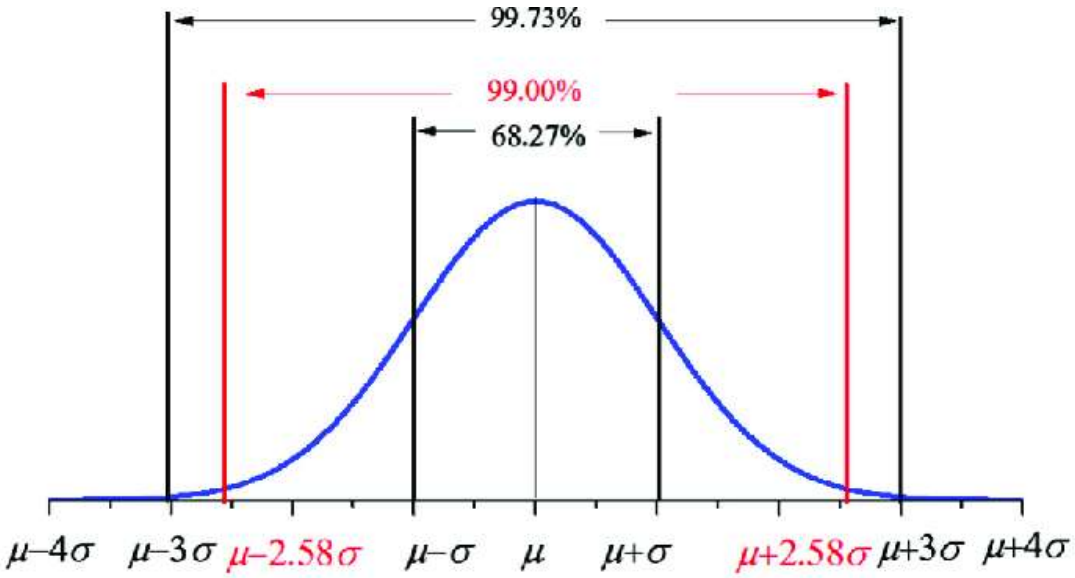
- Equivalence test (for Means)
- Multivariate analysis

Note : Inferential statistics can quantify uncertainty
– e.g. false positive rate alpha restricted to 5%, power for a given sample size & deviation from H0

Statistical approaches for comparative assessment using X-Sigma



Molecular weight distribution by size exclusion chromatography of 687 Copaxone® batches manufactured between 2005 and 2008.

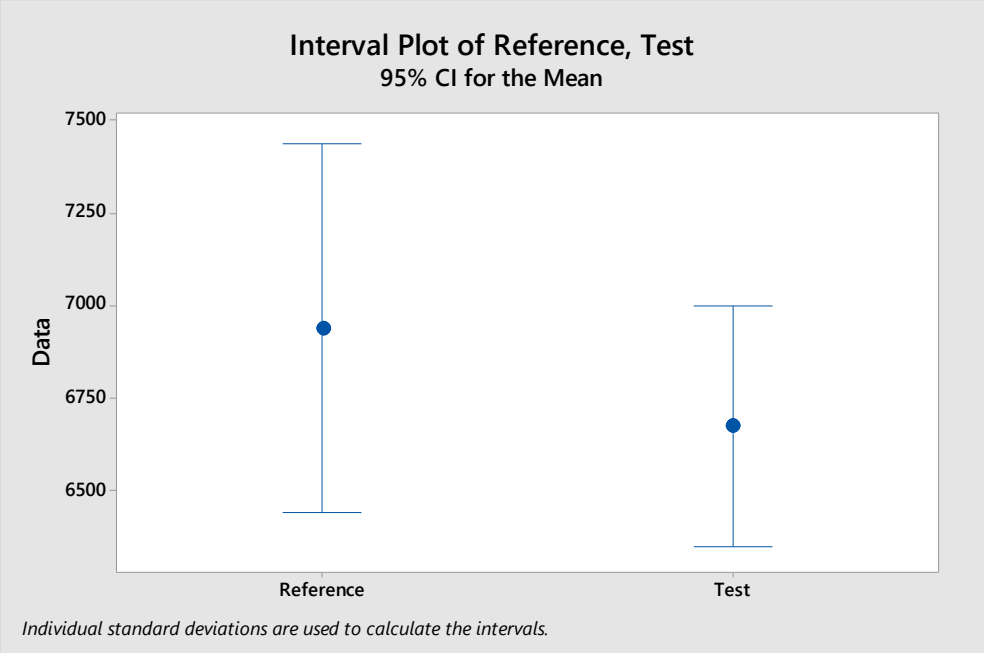


The data on Peak average molecular weight, Higher molecular weight at - 1SD, Lower molecular weight at +1SD of 687 batches of Copaxone® that are presented *by Innovator in the Citizen petition to USFDA dated 13th Nov-2009*

Tolerance interval

- If the test results are variable, then tolerance interval of the data can be used.
- A **tolerance interval** is a statistical **interval** within which, with some confidence level, a specified proportion of a sampled population falls. (Eg ; 95%, 99% CI)
- Rationale for Sample size (n): Risk based Approach
  Eg : 95% CI , 99% population
- Decision based on Coverage of Population and method uncertainty.

Equivalence test



Example: Average molecular weight comparison of test and reference product

p value is found to be more than 0.05,

The difference between the means is not statistically significant

Two-Sample T-Test and CI: Reference, Test

Method

μ_1: mean of Reference
μ_2: mean of Test
Difference: $\mu_1 - \mu_2$

Equal variances are assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Reference	10	6938	698	221
Test	7	6673	352	133

Estimation for Difference

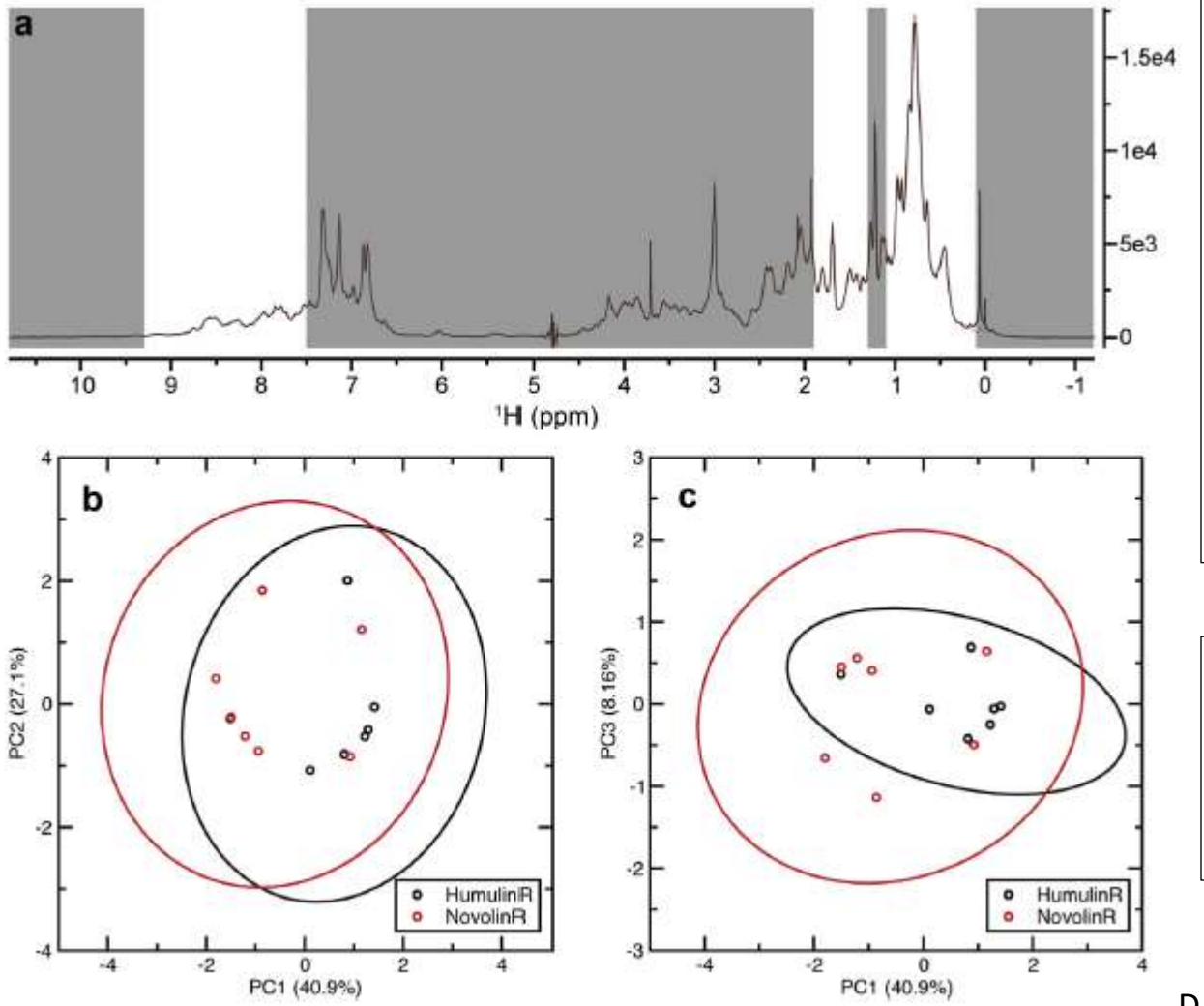
Difference	Pooled StDev	95% CI for Difference
265	585	(-349, 880)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.92	15	0.372

Multivariate analysis



Quantification of spectral difference for insulin human drug substances (DSs) from HumulinR® (black) and NovolinR® (red) in pH 7 buffer. The superimposed 1D 1H NMR spectra of the 2 representative lots of DSs are shown (a). Blinded spectral regions shown in gray were excluded in principal component analysis (PCA).

The PCA scores are plotted

between PC1 and PC2 (b) and between PC1 and PC3 (c). The 90% confidence ellipses are drawn for each brand of insulin human

Similarity scores for the observable HOS relevant signals were expressed in the unitless **Mahalanobis distance (D_M)** derived from PCA space with an unsupervised **chemometric approach**

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THANKS

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