Nanomedicine Pharmacokinetics and Bioanalytical Methods to Measure Drug Release

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Stephan Stern, Ph.D., DABT

NCL Nanotechnology Characterization Laboratory

NCI Alliance for Nanotechnology in Cancer

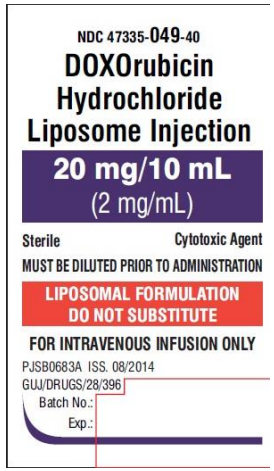
stephan.stern@nih.gov

http://ncl.cancer.gov

Nanomedicine Generics

The First Nanomedicine generic

- Sun Pharma's doxorubicin (DXR) HCl liposome, a generic version of Doxil, was the first generic nanomedicine approved by the FDA (2013).



Nanomedicines are complex formulations, and there will always be some degree of polydispersity and batch-to-batch variation. For generic versions, the challenge is to identify meaningful differences between the follow-on and the reference/innovator product.

More Nanomedicine generics are Coming

- Azaya has bioequivalence study underway now with a generic Doxil formulation, ATI-0918.
- Nantworks also has an ongoing bioequivalence study for a nab-paclitaxel alternative IG-001.

AZAYA THERAPEUTICS

NantWorks

As the number of FDA-approved nanomedicines continues to grow, the importance of developing a framework for evaluation of follow on versions of these treatments becomes increasingly important.

Nanomedicines ≠ Small Molecules, Biologics

API Identity is Known

Common Requirements for Approval of Generics

- Thorough PCC
- Bioequivalence studies

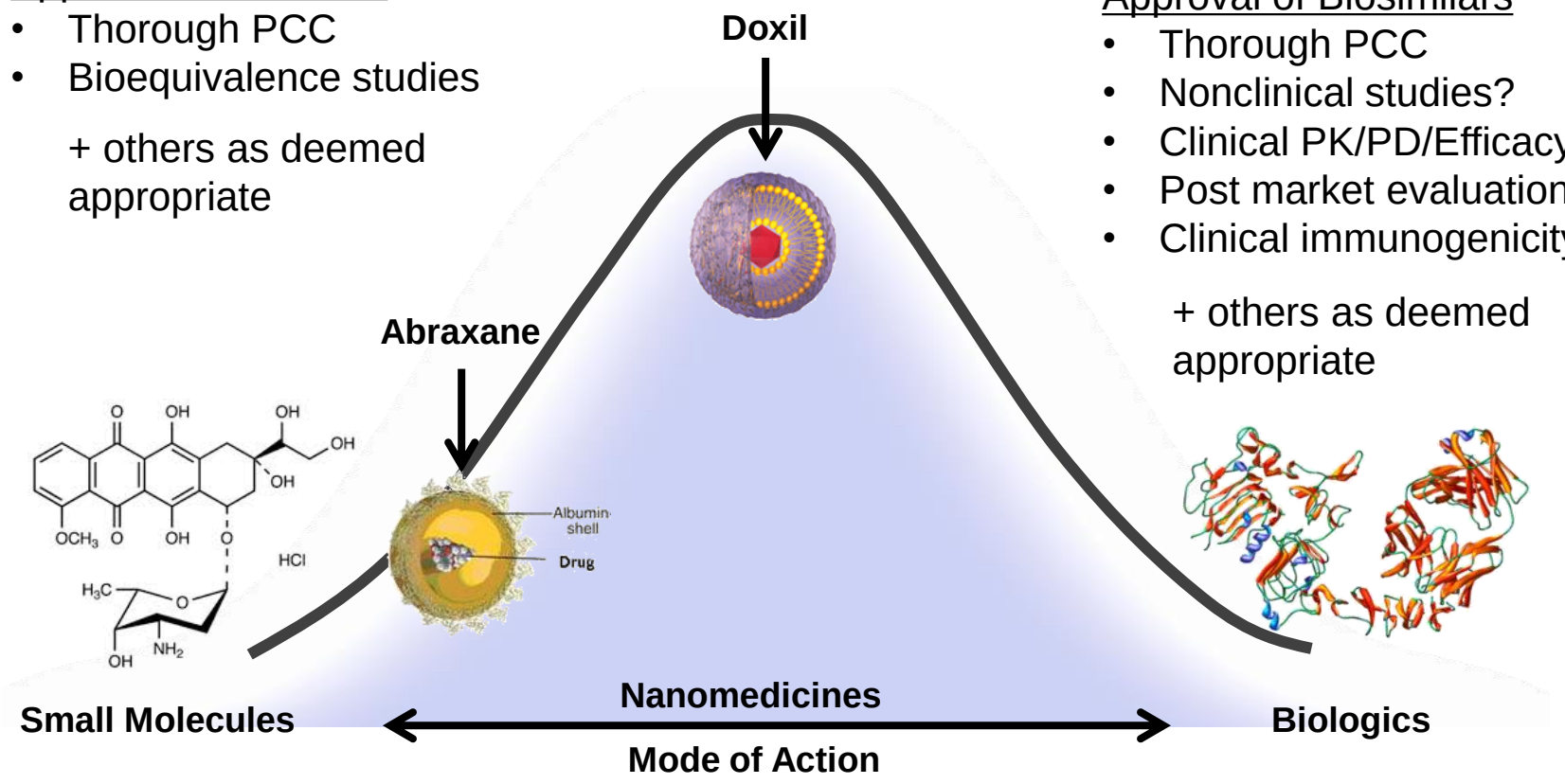
+ others as deemed appropriate

API Identity is a Complex Mixture

Common Requirements for Approval of Biosimilars

- Thorough PCC
- Nonclinical studies?
- Clinical PK/PD/Efficacy
- Post market evaluation
- Clinical immunogenicity

+ others as deemed appropriate



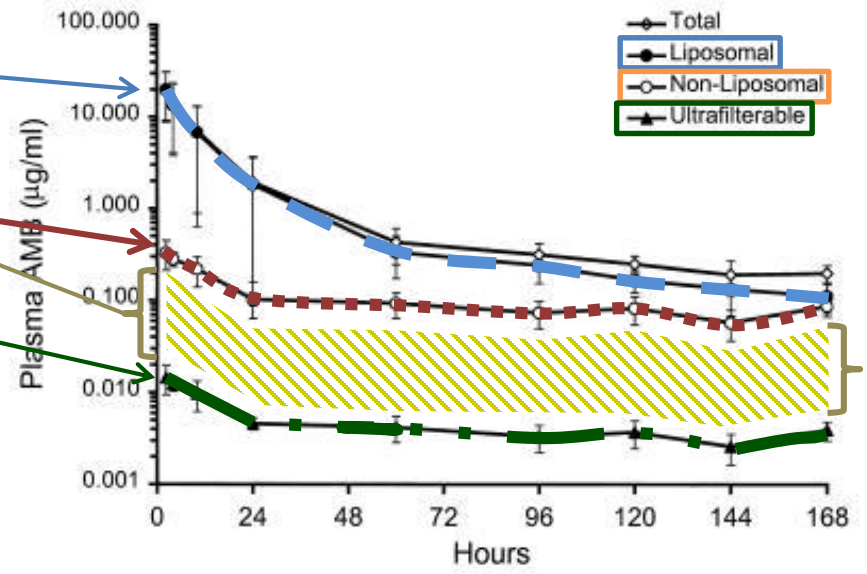
Nanomedicine Pharmacokinetics

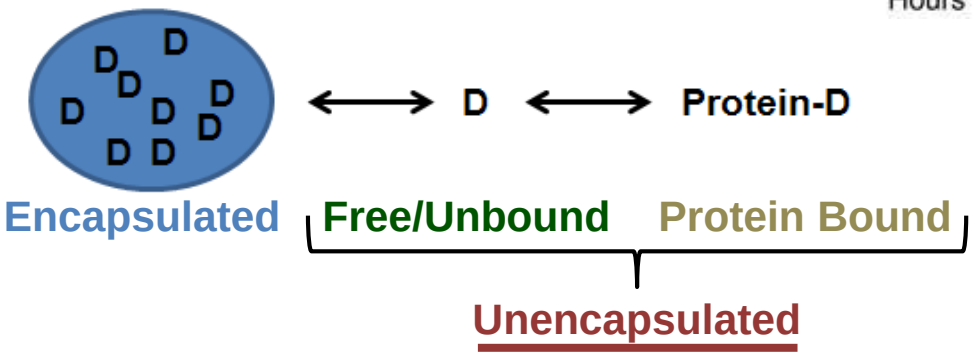
Nanomedicine drug fractions in circulation:

I. NM encapsulated fraction

II. Unencapsulated fraction

- 1-fu: protein bound fraction
- fu : unbound fraction

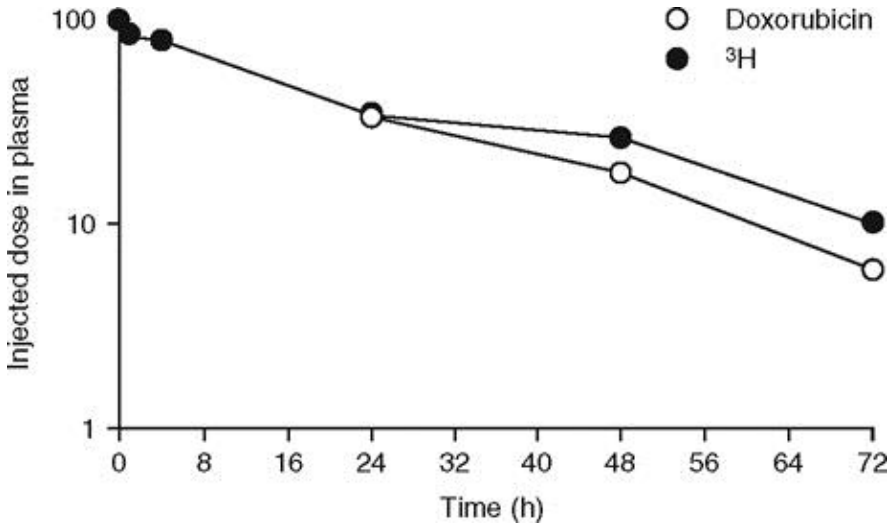




Bioequivalence studies require evaluation of drug release and unencapsulated drug fraction.

Bekersky et. al, *Antimicrob Agents Chemother* 2002, 46(3):834-40.

Case Study: Doxil "Stealth" Liposomes



Encapsulated $AUC_{0\text{-inf}}$ (μg x h/mL)	Unencapsulated $AUC_{0\text{-inf}}$ (μg x h/mL)
3,848	36

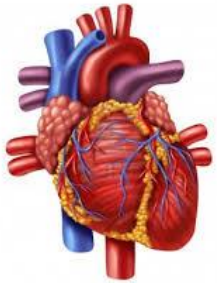
Radiolabel studies demonstrate slow release of encapsulated drug in mice

Encapsulated drug dominates clinical systemic profile

Liposome encapsulated DXR dominates the Doxil plasma profile, decreasing systemic free drug concentrations.

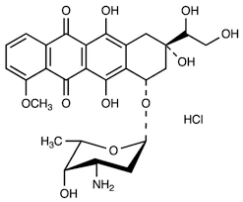
Clin Pharmacokinet 2003; 42 (5): 419-436

Distribution of Doxil "Stealth" Liposomes

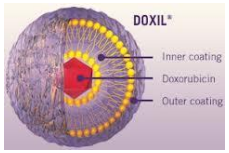


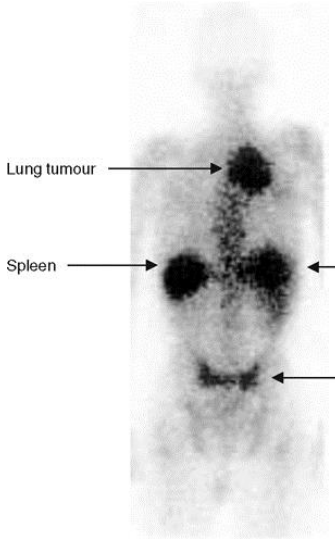
Heart
(Cardiotoxicity)

DXR HCl

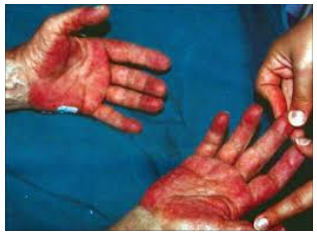


DXR





MPS Organs

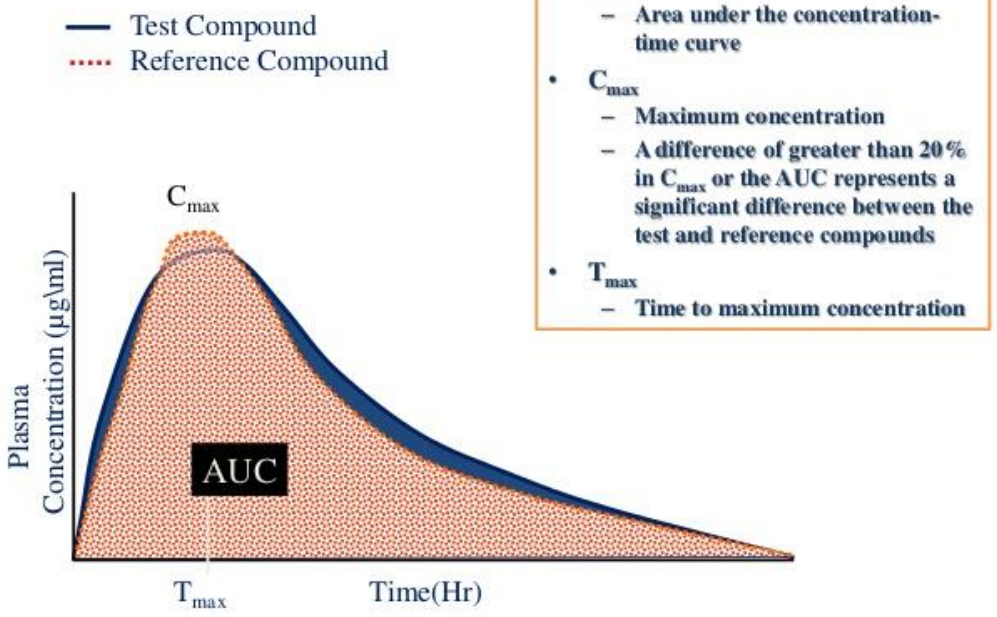


Palmar Plantar
Erythrodysesthesia

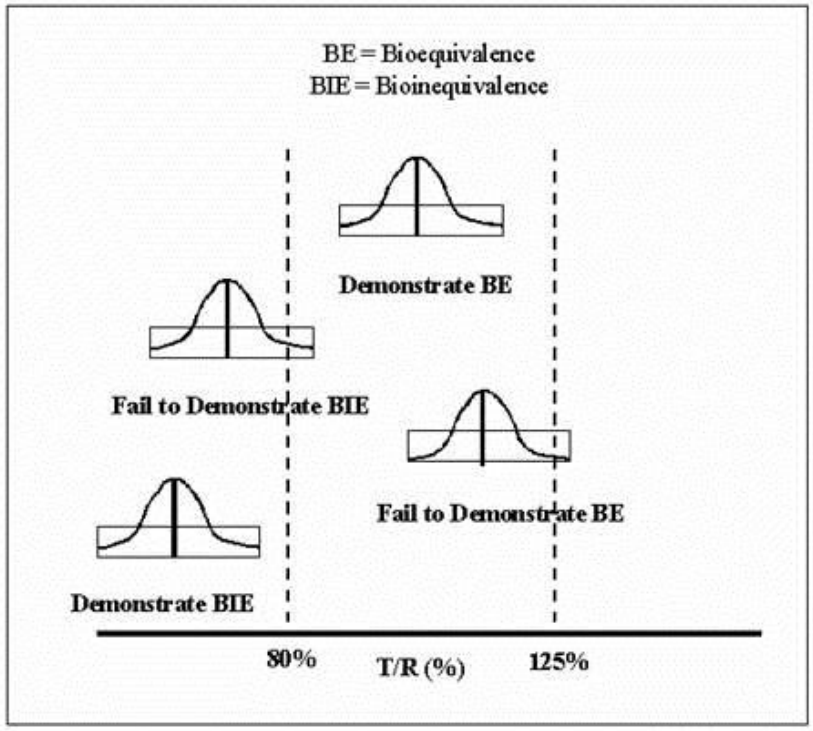
Doxil "Stealth" liposomes with encapsulated drug distribute primarily to MPS, but importantly also to tumor and skin.

Clin Pharmacokinet 2003; 42 (5): 419-436

Nanomedicine Bioequivalence



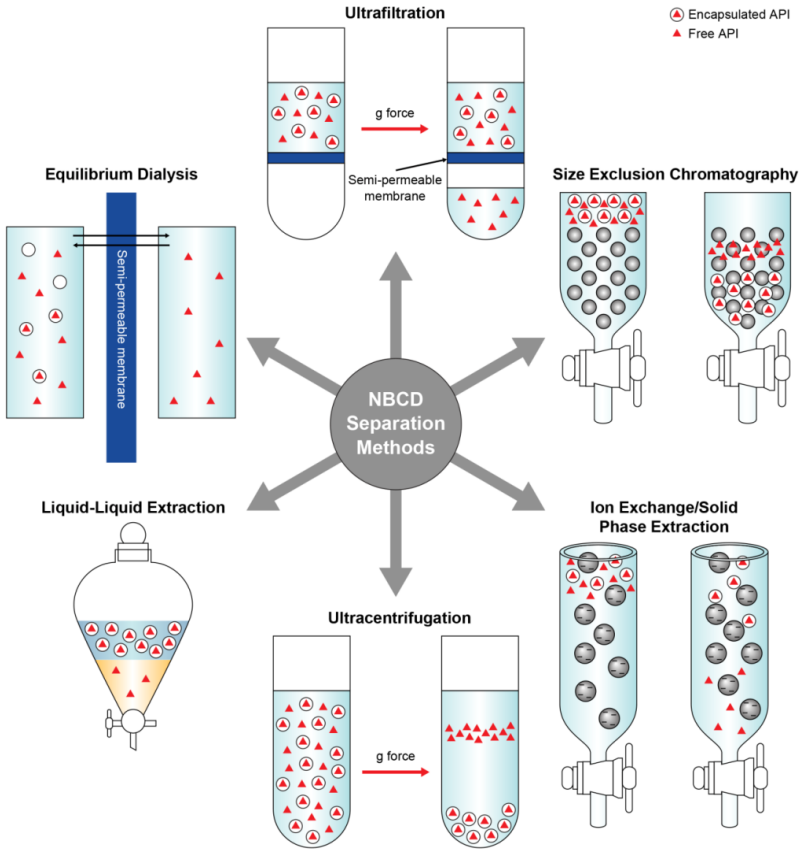
- **AUC**
  - Area under the concentration-time curve
- **C_{max}**
  - Maximum concentration
  - A difference of greater than 20% in C_{max} or the AUC represents a significant difference between the test and reference compounds
- **T_{max}**
  - Time to maximum concentration

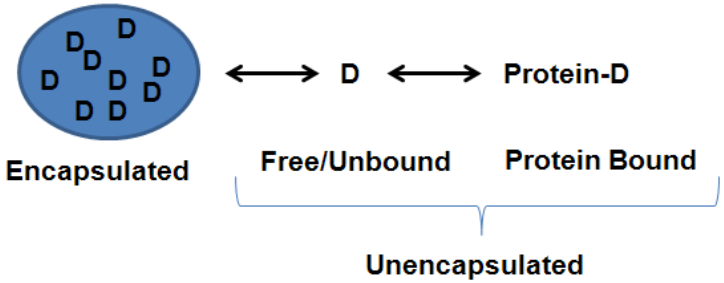


As per EMA/FDA guidance, nanomedicine bioequivalence is based on PK of total, unencapsulated and encapsulated drug fractions.

Ambardekar and Stern. NBCD Pharmacokinetics and Bioanalytical Methods to Measure Drug Release. In Daan Crommelin D and de Vlieger J (ed) Non-Biological Complex Drugs; the science and regulatory landscape. Springer, New York, NY; 2015.

Existing Fractionation Plasma Methods





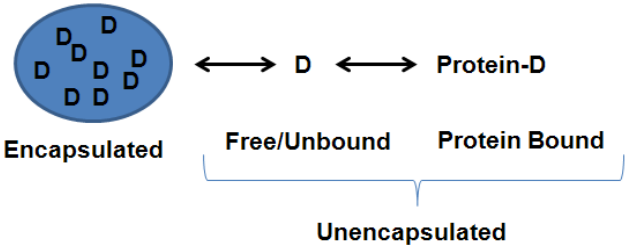
Main Problems

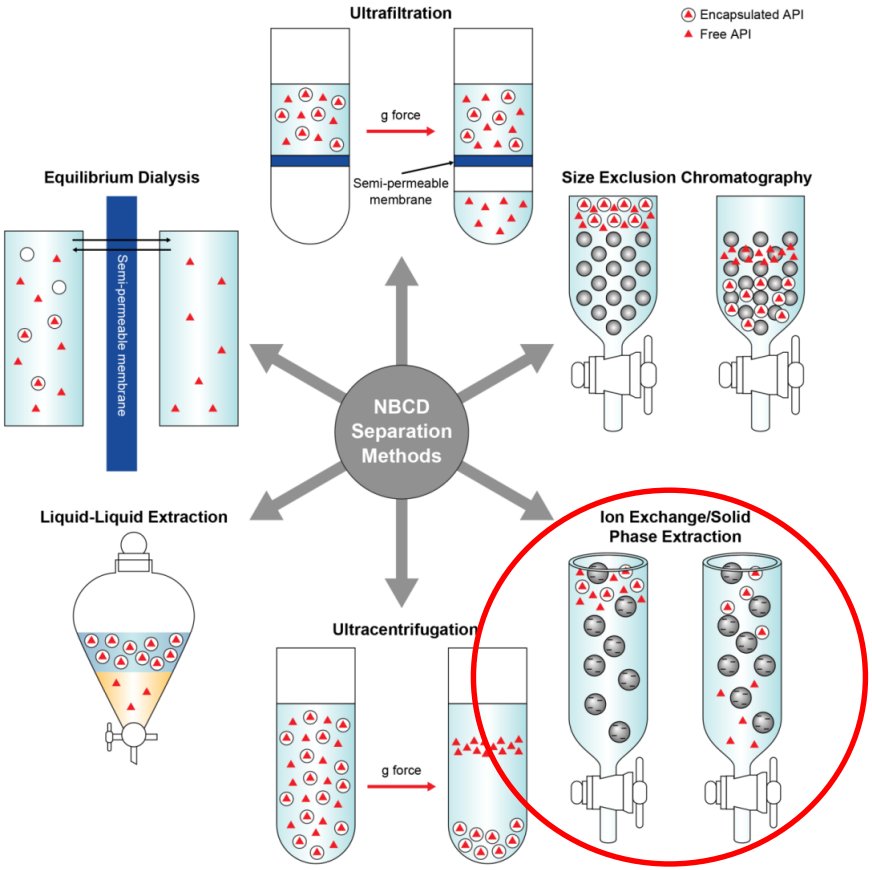
- Process induced artifacts
- Difficult to accurately differentiate protein bound and encapsulated API

Current methods have inherent flaws, adding inaccuracy and variability to nanomedicine fraction quantitation.

NBCD Pharmacokinetics and Bioanalytical Methods to Measure Drug Release. In Daan Crommelin D and de Vlieger J (ed) Non-Biological Complex Drugs; the science and regulatory landscape. Springer, New York, NY; 2015.

DXR HCl Liposome SPE Fractionation





Advantages

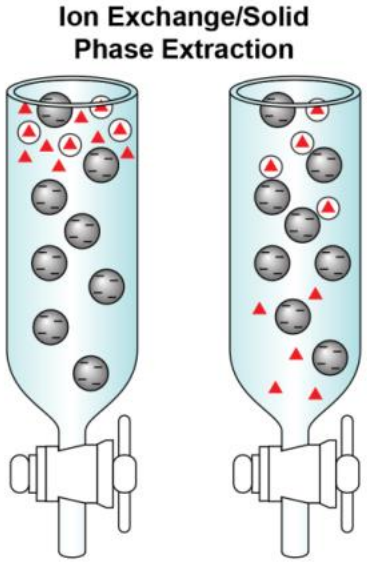
- Fast separation

Disadvantages

- Sample dilution
- Non-equilibrium conditions
- Process-induced drug release that can contaminate unencapsulated drug concentration

NBCD Pharmacokinetics and Bioanalytical Methods to Measure Drug Release. In Daan Crommelin D and de Vlieger J (ed) Non-Biological Complex Drugs; the science and regulatory landscape. Springer, New York, NY; 2015.

SPE Bioanalytical Method Validation



- Validation samples and standard curves are developed at low encapsulated:free drug ratios ~5:1 (e.g, 100:20 μg/mL to 50:10 ng/mL).
- Actual encapsulated:free drug ratios measured in patient samples are much higher: 100:1+!
- Process induced drug release is accounted for in the standards, but not unknowns.

BE Study Design Comparisons*

Company	Test Formulation	Reference Formulation	Dose	Patient Population	N
Sun Pharma	Generic DXR HCl Liposome	Caelyx (J&J)	50 mg/m^2	Advanced Ovarian Cancer	24
Company X	Generic DXR HCl Liposome	Caelyx (J&J)	50 mg/m^2	Advanced Ovarian Cancer	49-50

* Both studies are single-blind, randomized, two-way, cross over designs

European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP) (2011a) CHMP Assessment Report: Doxorubicin Sun.

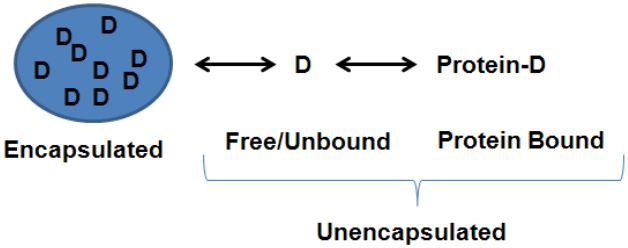
Discrepancies in Caelyx BE Trials

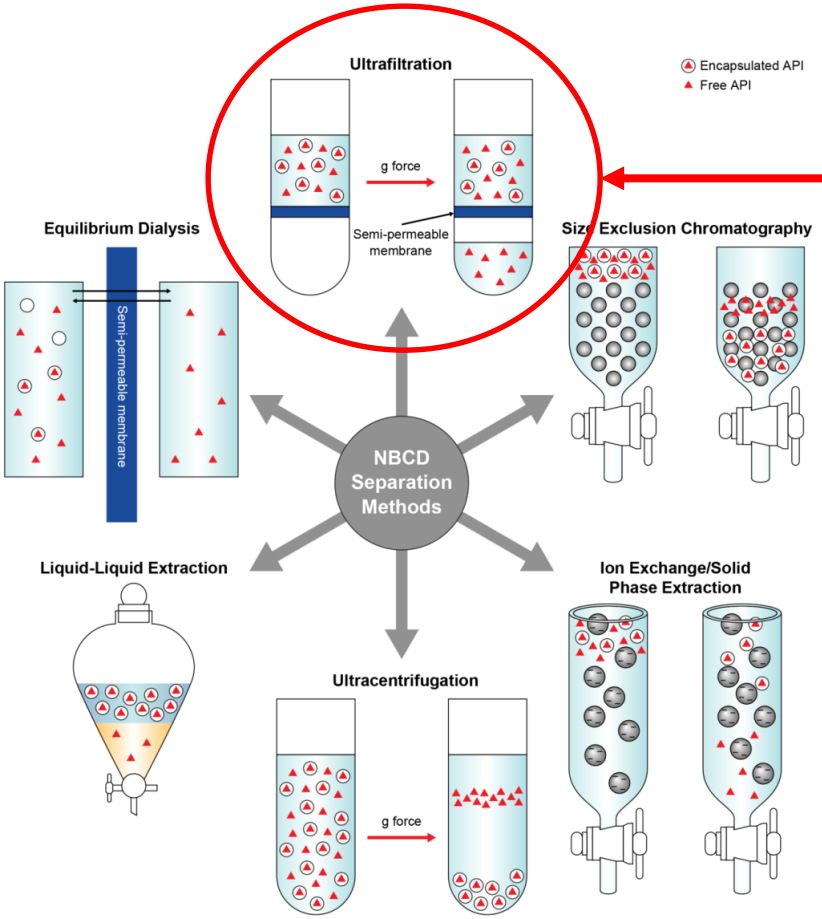
Company	Encapsulated $AUC_{0\text{-}inf}$ (mgxh/mL)	Unencapsulated $AUC_{0\text{-}inf}$ (mgxh/mL)	Encapsulated C_{max} (mg/mL)	Unencapsulated C_{max} (mg/mL)
Company X	5140	243	47	3.73
Sun Pharma	3,848	36	33	0.323
Fold difference	1.3x	6.75x	1.4x	11.5x

Current fractionation methods to measure unencapsulated drug do not appear accurate.

European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP) (2011a) CHMP Assessment Report: Doxorubicin Sun.

Improvement of Ultrafiltration Method





Advantages

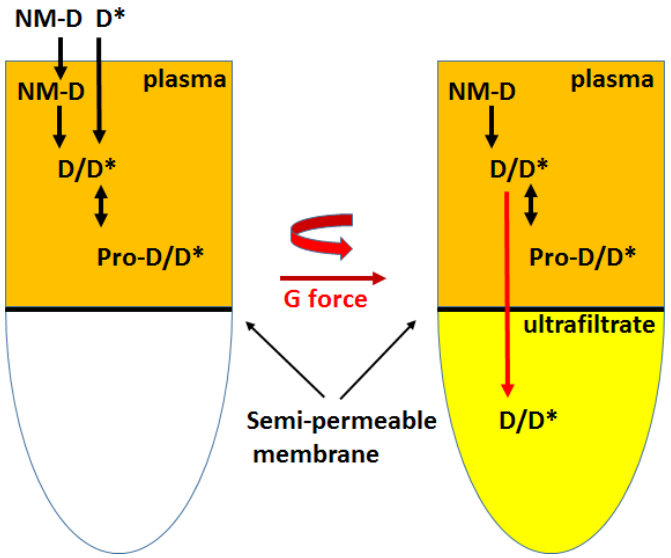
- free drug in equilibrium w/protein
- low encapsulated drug contamination
- fast separation
- no sample dilution
- less process-induced drug release?

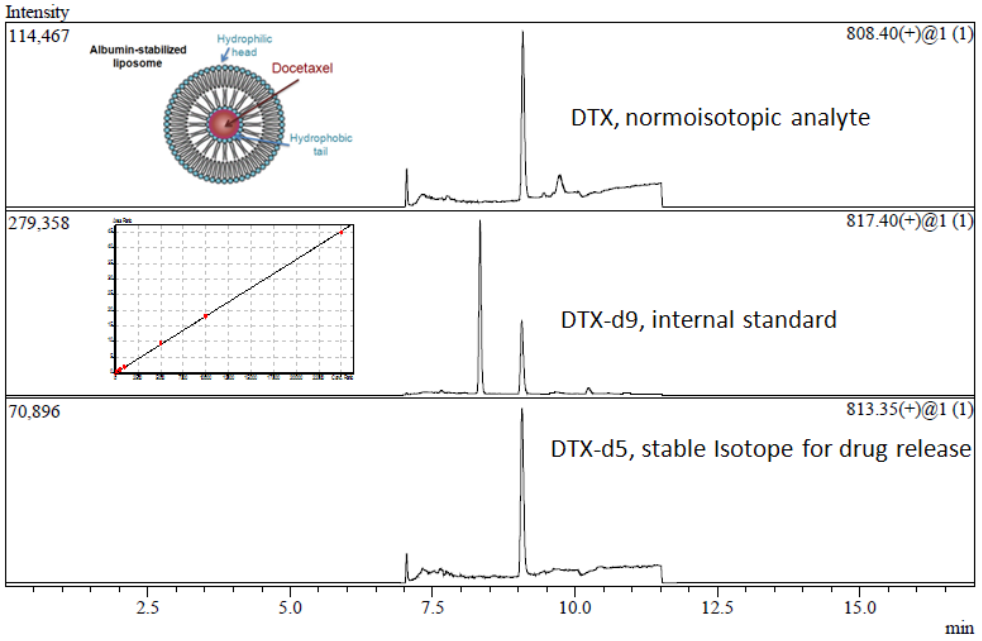
Disadvantages

- non-specific binding of API to filter membrane
- dissociation of the bound drug
- difficult to accurately differentiate protein bound and encapsulated API

NBCD Pharmacokinetics and Bioanalytical Methods to Measure Drug Release. In Daan Crommelin D and de Vlieger J (ed) Non-Biological Complex Drugs; the science and regulatory landscape. Springer, New York, NY; 2015.

Novel Stable Isotope Tracer Method to Measure Nanomedicine Drug Fractions



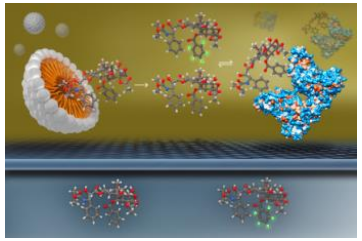


- The stable isotopically labeled drug (**D***) equilibrates with protein and unlabeled, normoisotopic drug (**D**) released from nanomedicine (NM) formulation.
- % **D***bound estimation gives reliable prediction of %**D** bound.

%Bound = $\frac{([\text{Total D*}] - [\text{Ultrafilterable D*}]) * 100}{[\text{Total D*}]}$

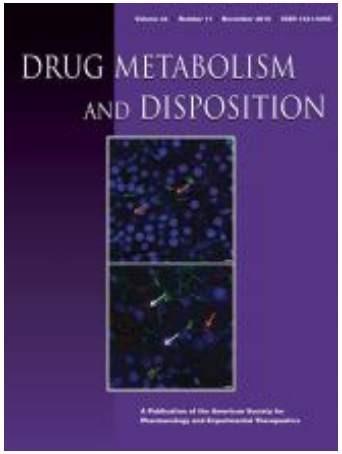
[Unencapsulated D] = $\frac{[\text{Ultrafilterable D}]}{(1-(\%\text{Bound D*}/100))}$

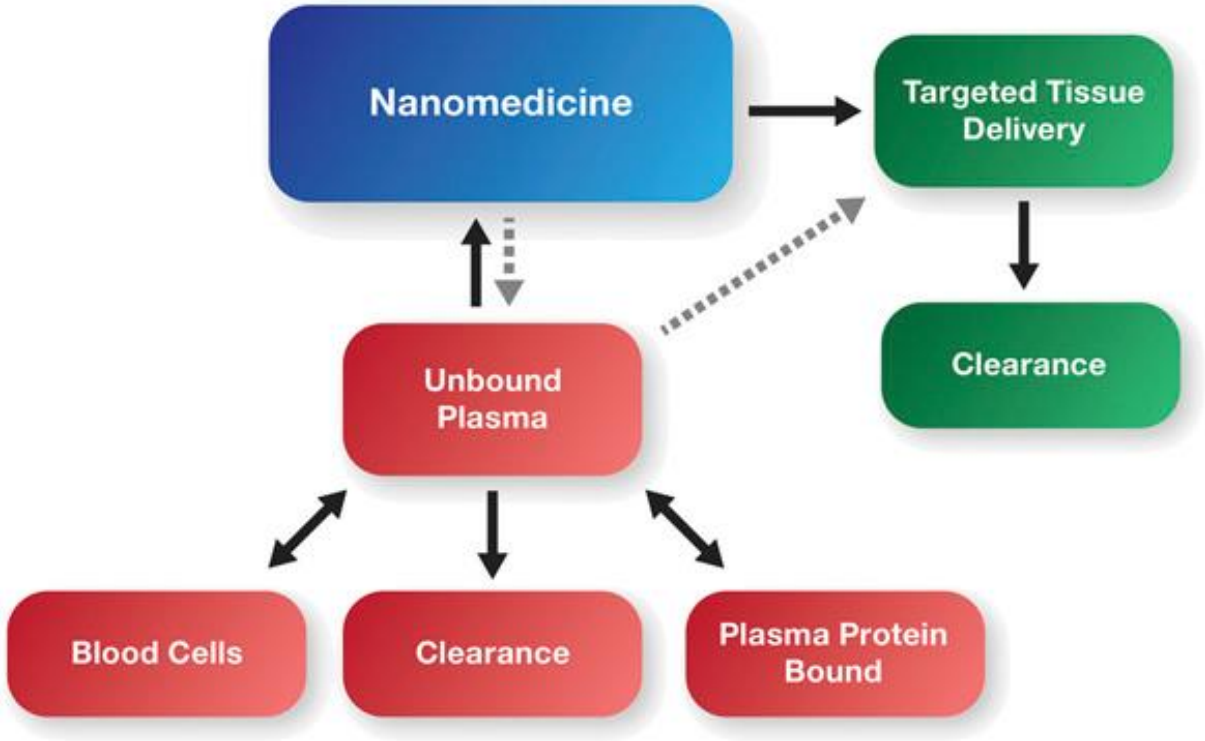
[Encapsulated D] = [Total D] – [Released D]



Cover Article: J Control Release, 2015, 220(Pt A):169-74.

Commentary: When is it Important to Measure Unbound Drug in Evaluating Nanomedicine Pharmacokinetics?

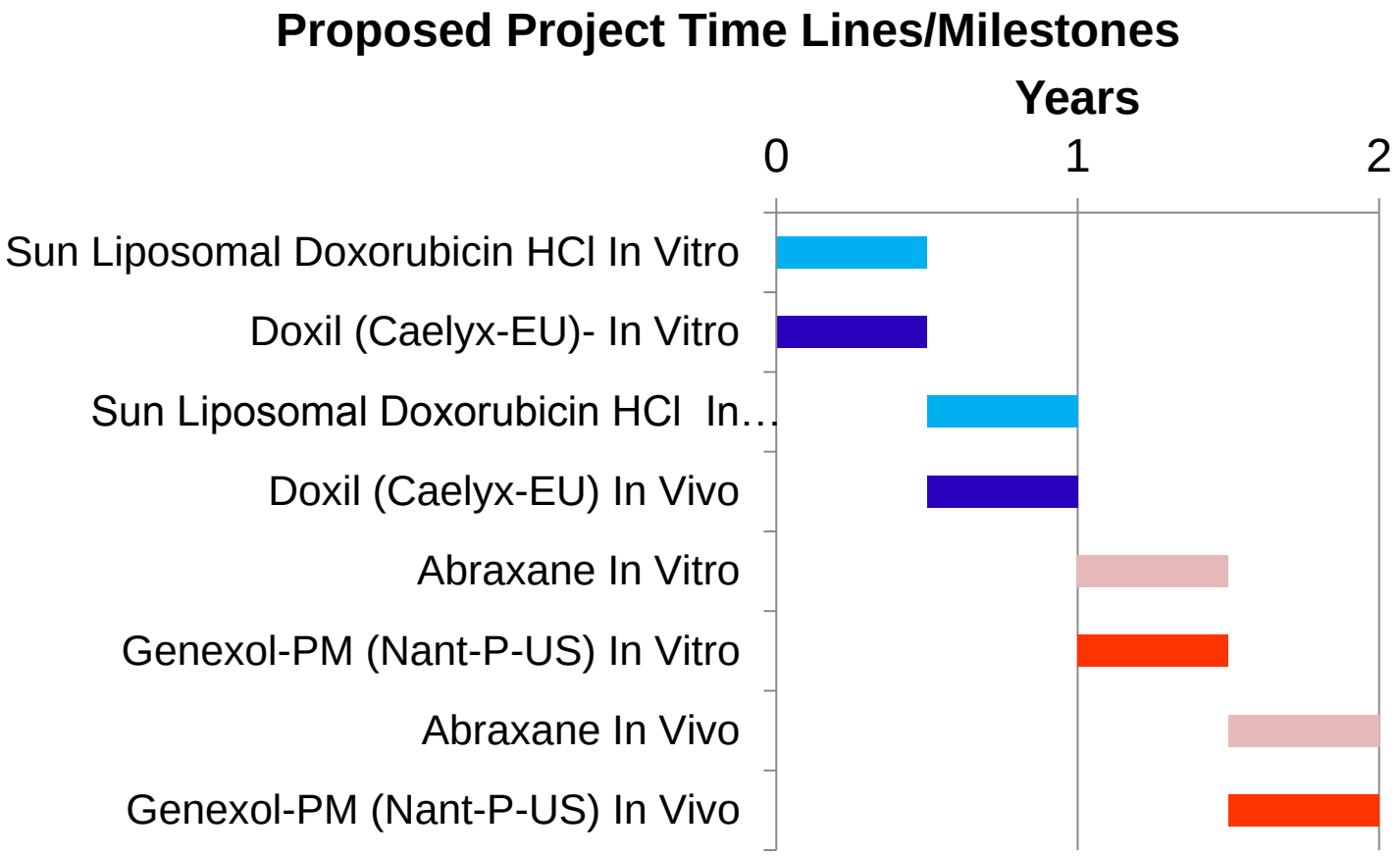




- **When unbound drug is in equilibrium with the formulation bound drug, and unbound drug fraction may change as a function of formulation (e.g. micellar systems)**
- **When unbound drug is the unencapsulated drug, e.g. Abraxane®**

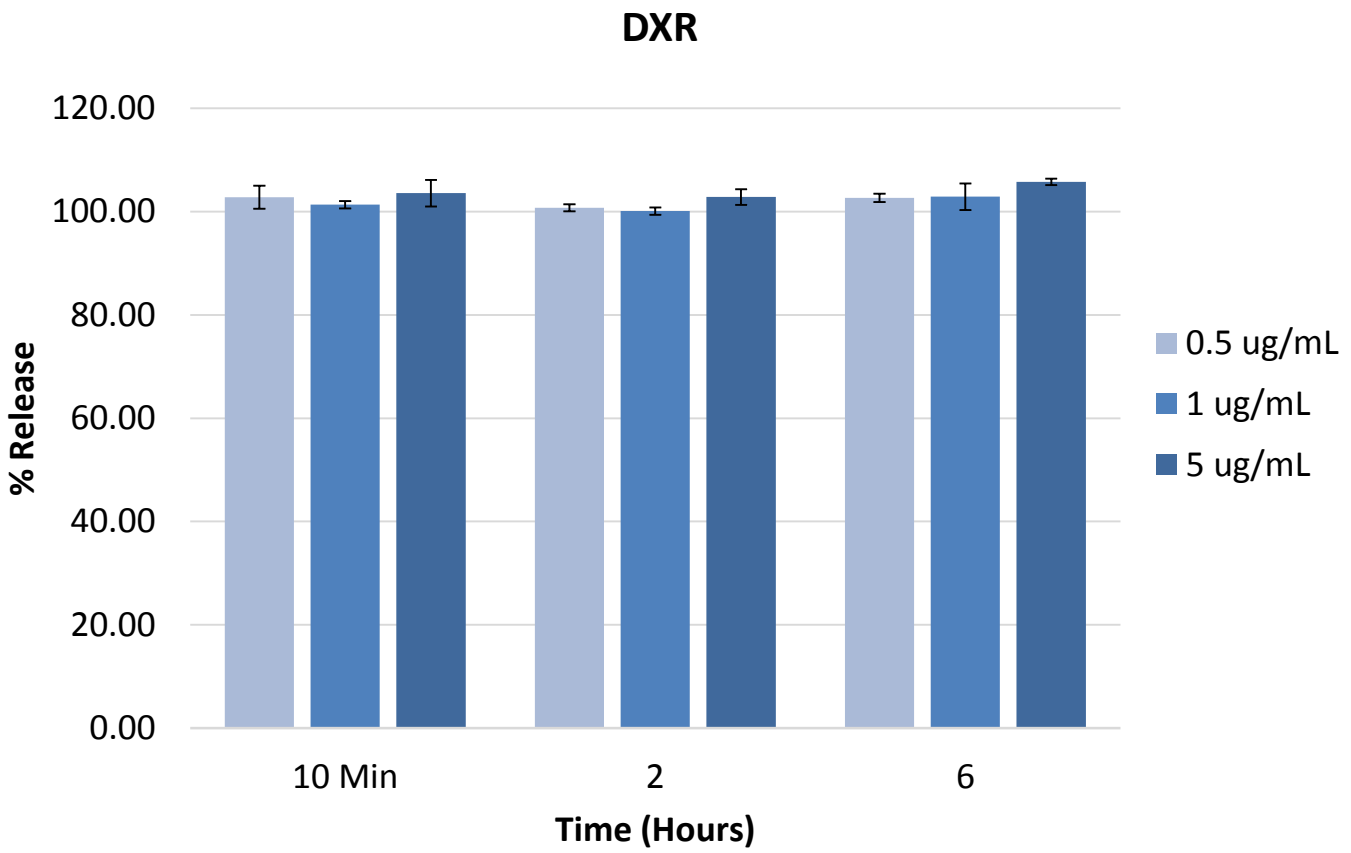
Drug Metab Dispos 44:1–6, December 2016

FDA-NCL Interagency Agreement: Evaluation of Stable Isotope Tracer Method



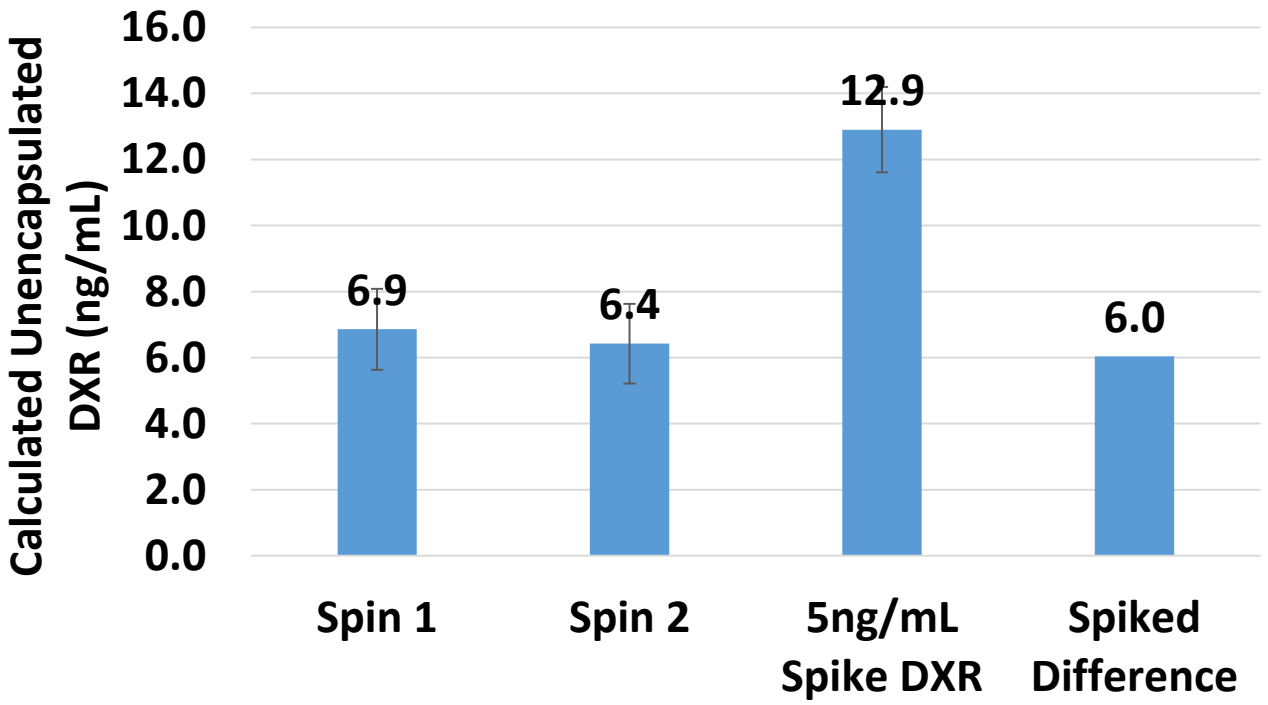
Interagency agreement to evaluate the stable isotope tracer method for determination of generic nanomedicine bioequivalence.

Free DXR Control in Rat Plasma



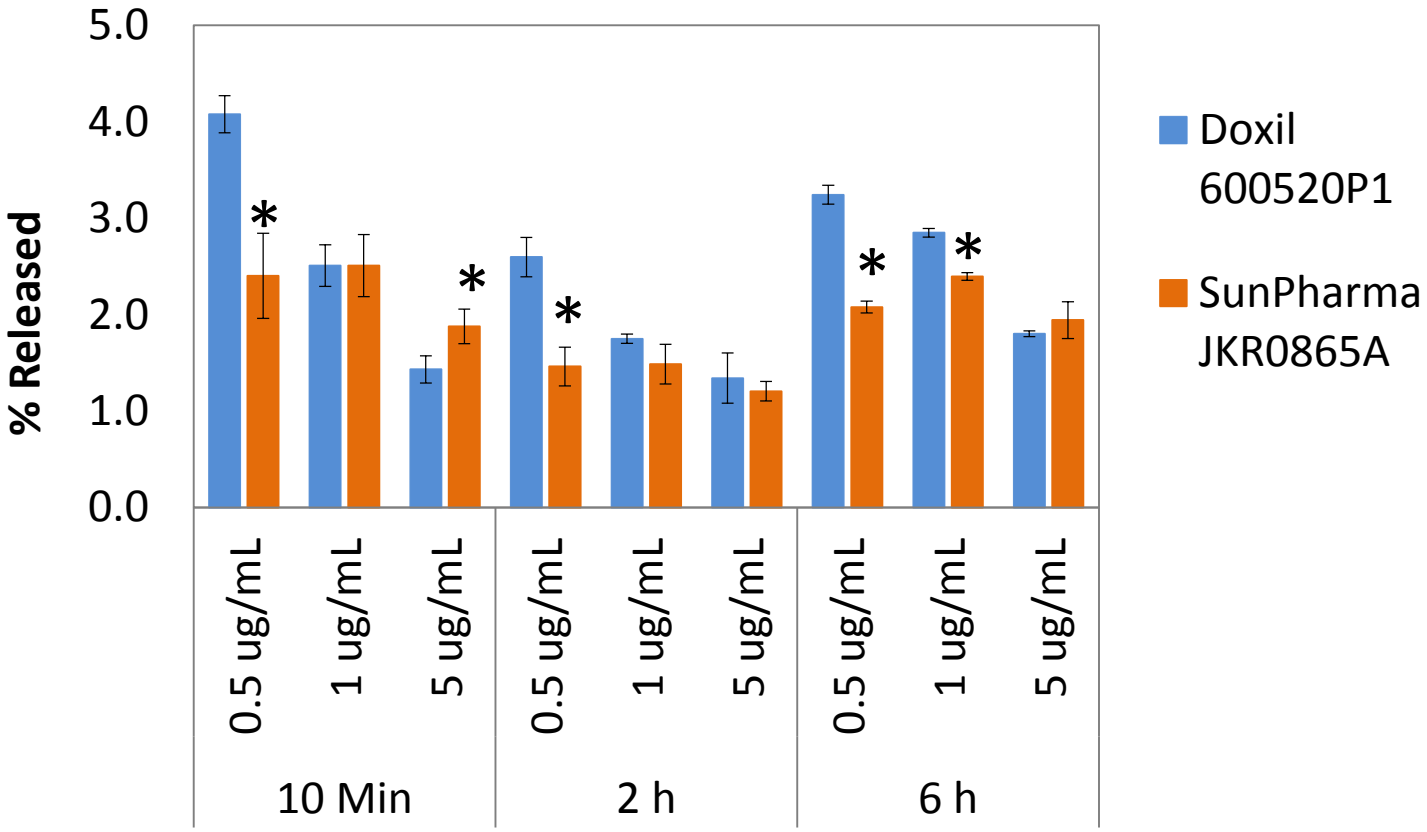
Free DXR was recovered within 10% of theoretical, with CV<5%.

Process and Spike Controls - Results



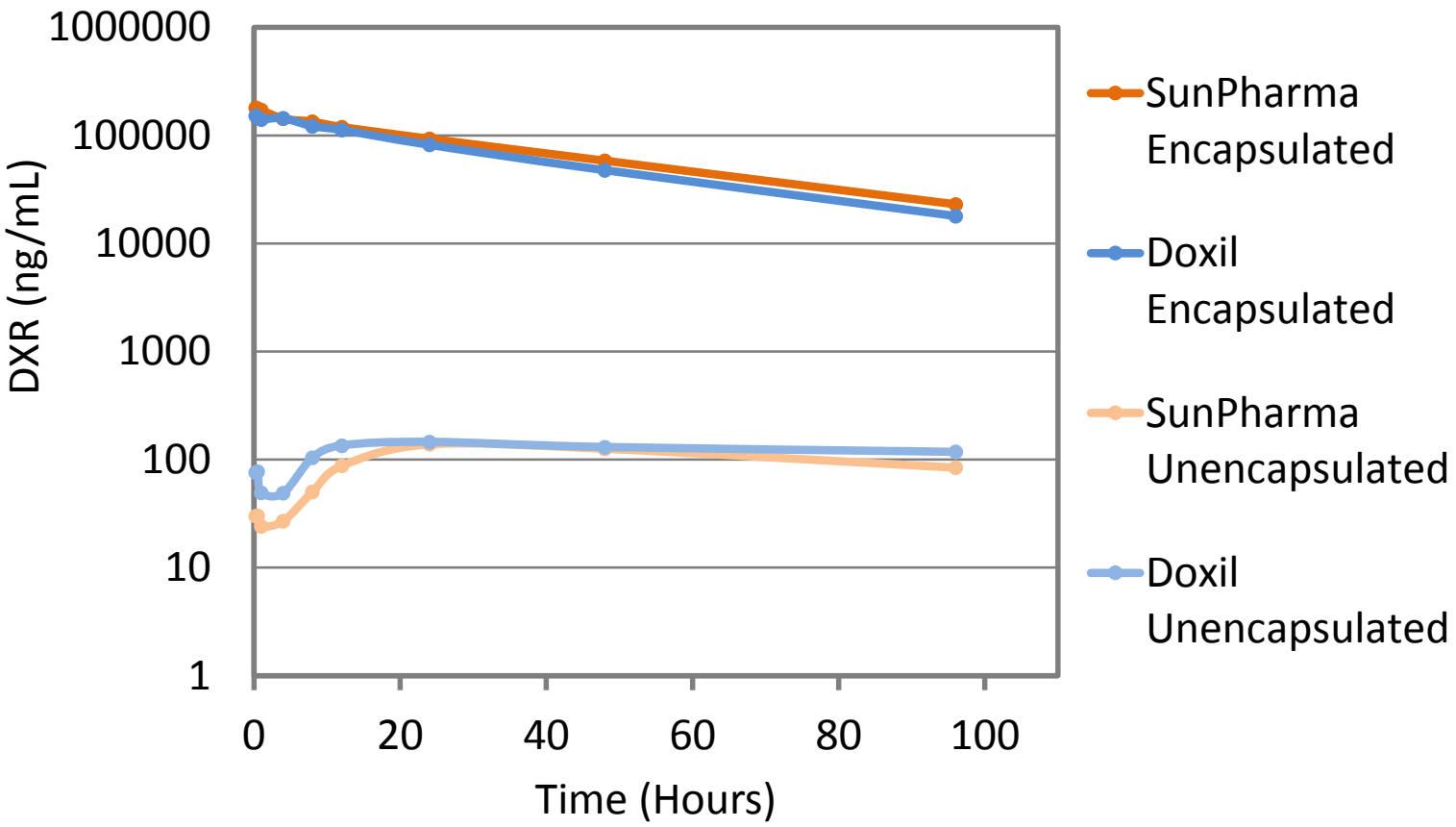
- Samples tested at an encapsulated:free DXR drug ratio of 1000!
- Double processing (spin) did not alter the unencapsulated DXR estimate
- The 5 ng/mL spike recovery was within 20% of theoretical

In Vitro Drug Release in Rat Plasma



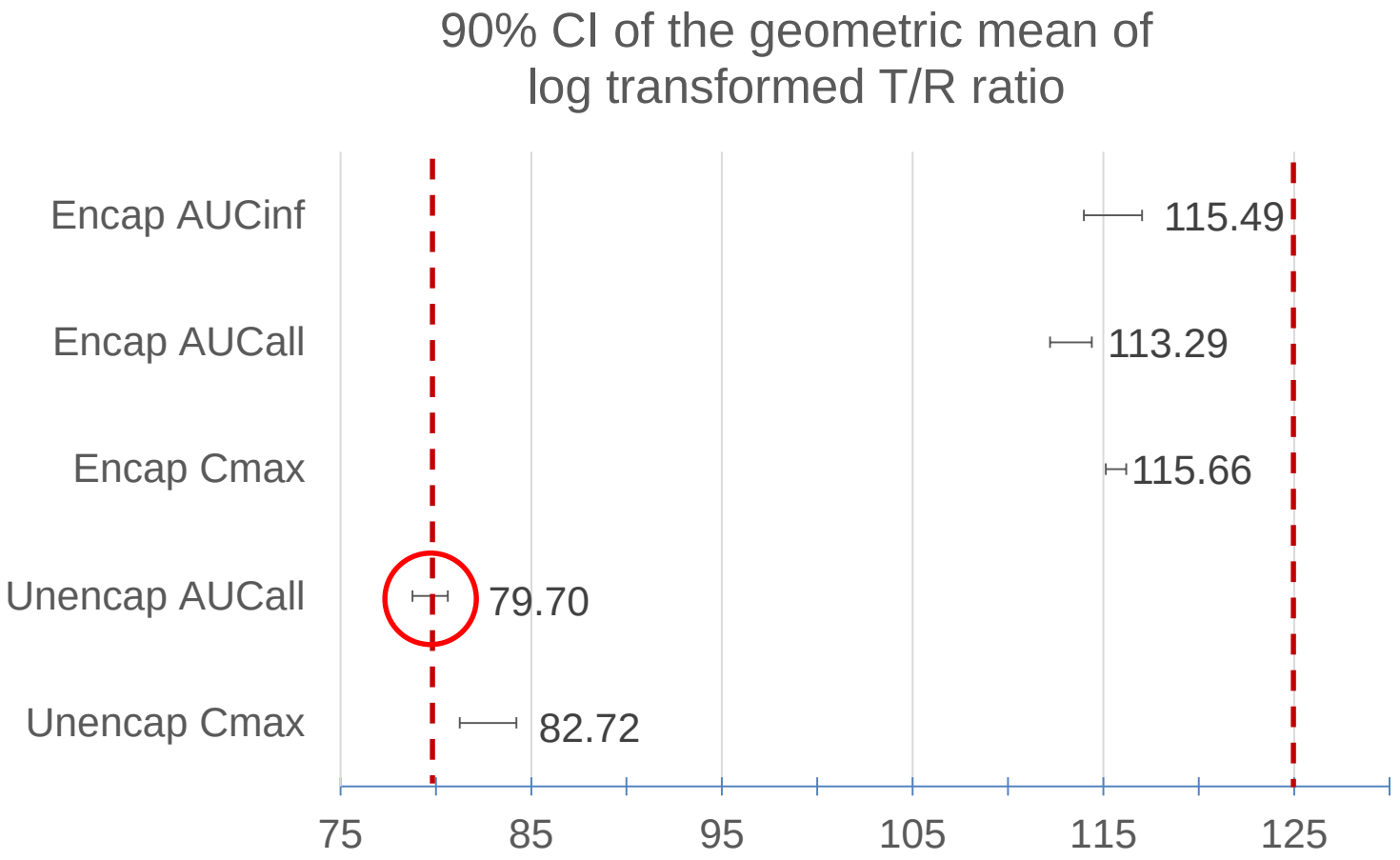
Drug release for the two liposomal products were similar, ~2%.

Liposomal DXR Pharmacokinetics in SD Rats



Unencapsulated and encapsulated DXR profiles for the two liposomal products were similar.

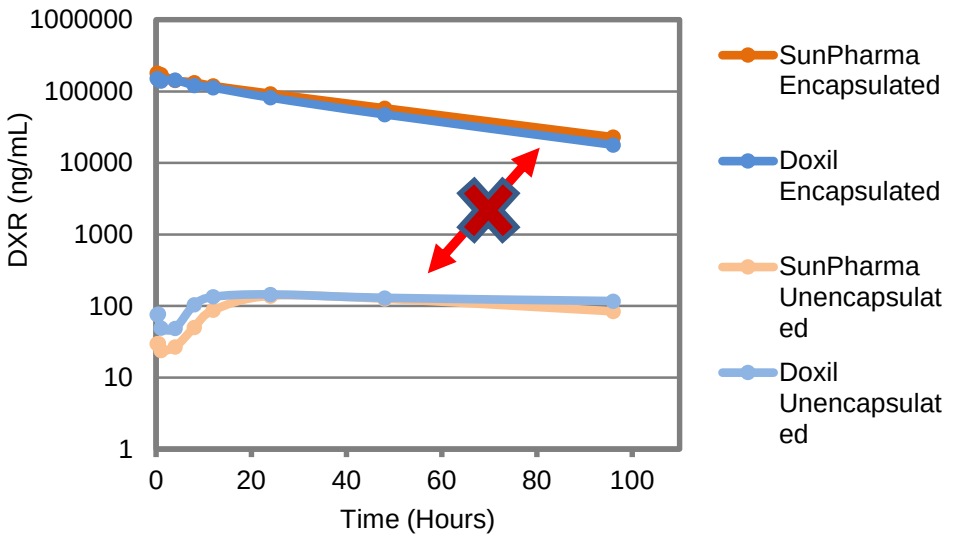
TOST Bioequivalence Analysis



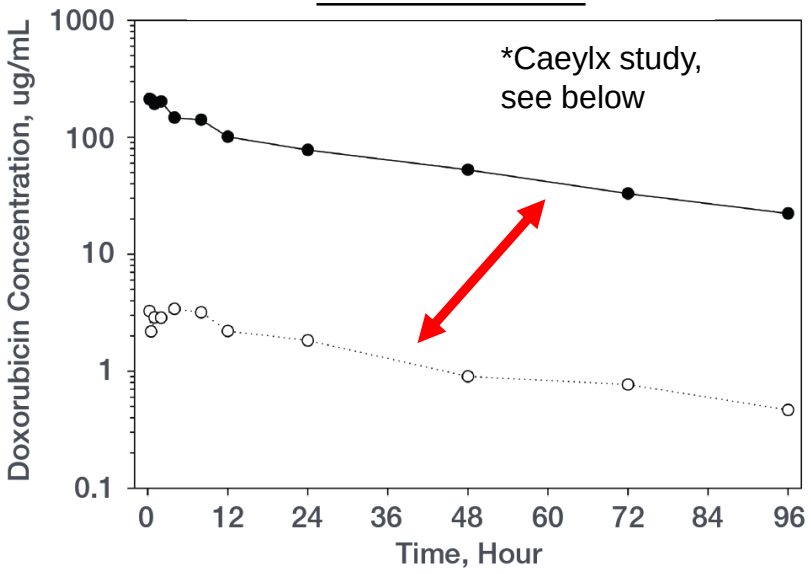
All PK parameters found to be equivalent, with 90%CI of the test (Sun Pharma)/reference (Doxil) ratio within 80-125% by TOST, except for unencapsulated AUCall.

Comparison of Stable Isotope vs. SPE Methods

Stable Isotope Method



SPE Method



- **Important differences for stable isotope versus previous literature SPE methods:**
  - While encapsulated drug profiles were identical, unencapsulated drug concentrations are much lower (10-18 fold!)
  - Slope of terminal phase for unencapsulated drug is much flatter, and does not paralell the encapsulated drug profile
  - Tmax is much later 33h vs ~4h, for stable isotope vs. SPE, respectively

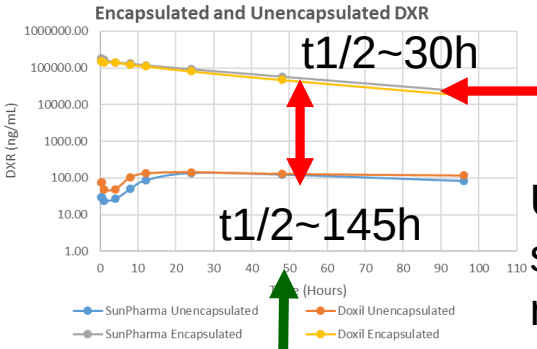
* Caeylx study in rats at 6 mg/kg i.v. bolus, Azaya Therapeutics, AAPS abstract 2013
** Caelyx study in male SD rats 10 mg/kg i.v. bolus, Sun Pharma, Cancer Chemother Pharmacol (2017) 79:899-913

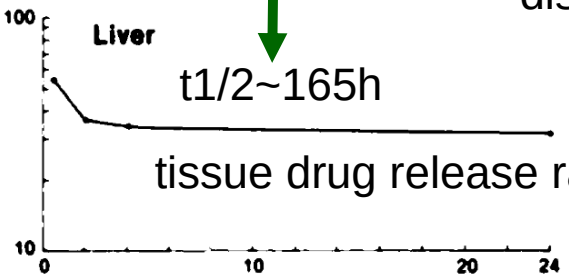
Comparison of Stable Isotope vs. SPE Methods

Which unencapsulated drug profile is more reasonable, stable isotope or SPE method?

- SPE estimated unencapsulated drug t1/2 is not reasonable……..

Stable Isotope Method



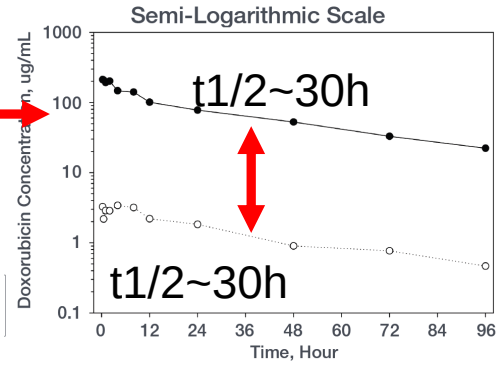


tissue drug release rate

tissue distribution rate

Unencapsulated t1/2 should mimic tissue drug release rate, not tissue distribution rate

SPE Method



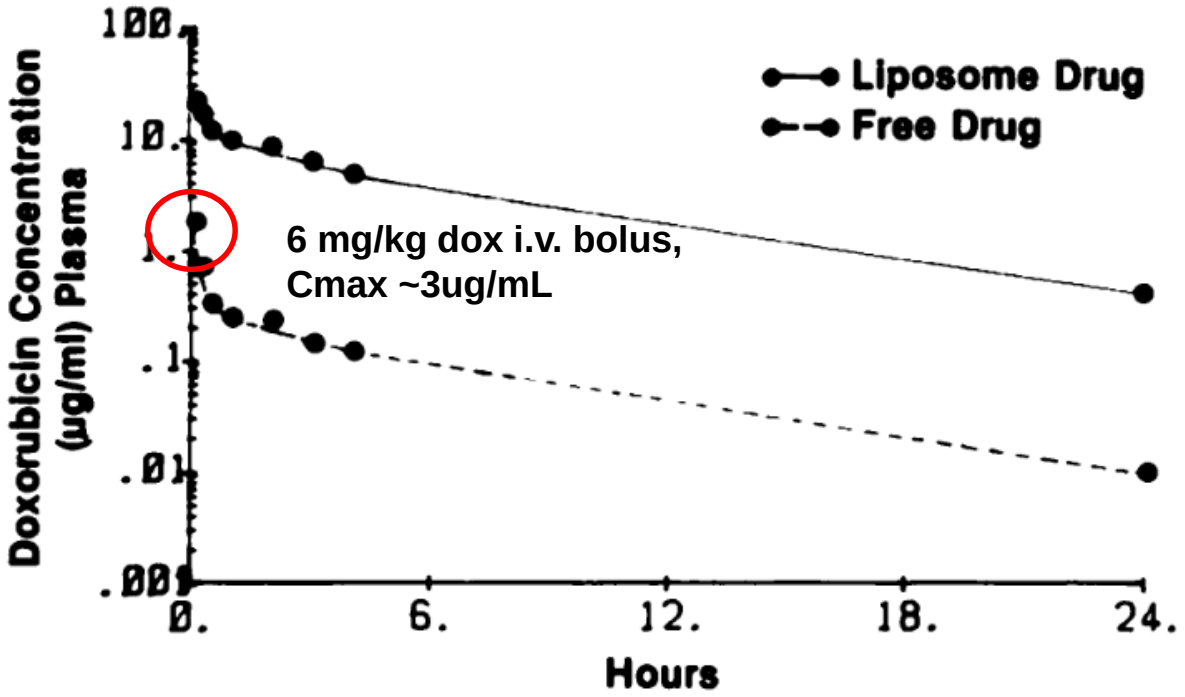
Liposomal drug release in tissue is similar to the stable isotope estimated t1/2…

Cancer Res. 1986 May;46(5):2295-9.

Comparison of Stable Isotope vs. SPE Methods

Which unencapsulated drug profile is more reasonable, stable isotope or SPE method?

- SPE estimated Cmax is not reasonable……..



Impossibly, SPE estimated unencapsulated DXR profiles have the same Cmax values as i.v. bolus rat studies of free, non-liposomal DXR, ~1-3 ug/mL.

Cancer Res. 1986 May;46(5):2295-9.

In Summary

- The lack of robust nanomedicine fractionation methods are an impediment to both nanomedicine characterization and nanomedicine generic development.

- Higher quality pharmacokinetic data will decrease patient sample size and facilitate regulatory determination of bioequivalence.

- The NCI, in collaboration with the FDA, is supporting development and validation of highly accurate and precise nanomedicine fractionation methods.

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Director

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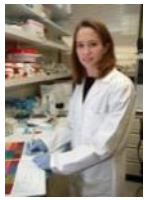
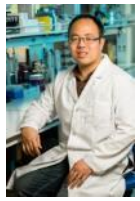


Sarah Skoczen, M.S.

Kelsie Snapp, B.S., M.B.A.

Immunology

Marina A. Dobrovolskaia, Ph.D., M.B.A., PMP



Enping Hong, Ph.D.

Ankit Shah, Ph.D.

Barry W. Neun, B.S.

Ed Cedrone, B.S.

Physicochemical Characterization

Jeffrey D. Clogston, Ph.D.

Jiewei Wu, Ph.D.

Yingwen Hu, Ph.D.

Sonny Man, M.S.

Alison Vermilya, M.S.

Cassandra Mankus, B.S.

Cancer Biology

Pavan Adiseshaiah, Ph.D.

Bhawna Sharma, Ph.D.

Timothy M. Potter, B.S.

Travis Kerr, M.S.

Alliance Management

Jennifer Grossman, Ph.D.

Rachael M. Crist, Ph.D.

Maggie Scully, Ph.D.

Support/Admin.

Christopher B. McLeland, B.S., M.B.A.

Jamie Rodriguez, B.S.

Becky Schneider, B.S.

Supporting Labs

- Laboratory of Animal Science Program
- Pathology/ Histotechnology Lab
- Electron Microscopy Lab

Contact Info:

Stephan Stern

(301) 846-6939

Stephan.stern@nih.gov

http://ncl.cancer.gov

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