Novel Method for Extraction of Free Drug from Nanoparticle Formulation in Biological Matrix

Poster Presentation by: Sarah L. Skoczen, Stephan T. Stern, Scott E. McNeil, Nanotechnology Characterization Laboratory, Advanced Technology Program, SAIC-Frederick, Inc., National Cancer Institute-Frederick, Frederick, Maryland

HIGHLIGHTS: High extraction efficiency

RP-XTR

INTRODUCTION

There is currently no consistent, robust method for separating free and nanoparticle-bound drug from a biological matrix. This method of free drug extraction is urgently needed in order to predict in vivo stability and determine bio-available, free drug profile in pharmacokinetic samples. These methods could aid in the design of nanoformulations with better controlled release properties, as well as aid in safety assessment and estimation of a clinical starting dose. This study utilizes a Dispersive Pipette XTRaction technology for INTip™ solid phase extraction of free doxetaxel (DTX) and paclitaxel (PTX) from two nanoformulations. DTX was extracted from a nanoliposomal formulation (Azaya Therapeutics) and PTX was extracted from an albumin nanoparticle formulation (Abraxane™, Abraxis BioSiences, Inc.), both in 25% rat plasma. Extracted drug was analyzed by a validated HPLC method and extraction efficiency was consistently 70% for both drugs. Consistent with the pharmacokinetic data for these formulations, neither formulation was found to be stable, with drug readily extracted from the nanoformulation containing biological matrix. Chemical analysis of nanoliposomal cholesterol and albumin nanoparticle protein in the extraction flow through suggested that the nanoformulations themselves were not extracted. Further size analysis by dynamic light scattering of the flow through from saline extracted samples demonstrated that the nanoliposome size was unaffected by the sample extraction procedure, while the albumin nanoparticle was disrupted. The data supports further investigation of this method for extraction of free drug from biological matrix.

MATERIALS AND METHODS

1 mL XTR tips with Reverse Phase (RP) sorbent were purchased from DPX Technologies, LLC (Columbia, SC). Borosilicate glass tubes, 12.75 mm were purchased from Fisher Scientific (Pittsburgh, PA). BSA and Acetonitrile, gradient grade for HPLC were purchased from Sigma (St. Louis, MO). Rat plasma was purchased from Innovative Research (Novi, Michigan). Paclitaxel (PTX) (Catalog # P-9600) and docetaxel (DTX) were purchased from LC Laboratories (Woburn, MA). HPLC sample 96 well V-bottom plate were purchased from Perkin Elmer (Waltham, MA). Silicone sealing mat were purchased from Axygen (Union City, CA).

Sample Preparation:

RP-XTR tips were conditioned by aspirating 250 µL of acetonitrile (ACN), and dispensing to waste. Next aspirate and dispense DI water. The tips were then blocked to prevent nonspecific extraction of the nanoparticle by aspirating 250 µL of 2% BSA, then dispensing to waste 2 times. Wash by aspirating and dispensing with DI water.

Figure 1. 1 mL XTR pipette tip with Reverse Phase (RP) sorbent.

Aspirate 250 µl of the sample into the blocked tip, retain for 30 seconds, and then dispense to waste. Next, aspirate 250 µl of DI water and dispense to waste. Aspirate and dispense 2 times with 250 µl of 100% ACN to mix, and then dispense final eluant to a collection tube. To remove any protein that may be in the sample, the ACN extracts were further diluted with ice-cold ACN in a 1:5 ratio, then frozen at -80°C for 10 minutes, and thawed and centrifuged for 20 minutes at 14kg. The supernatant was transferred to a glass borosilicate tube, and dried under nitrogen at 48 °C in a TurboVap®. The dried sample residues were reconstituted in 500 µL of 50/50 ACN-DI water and analyzed by HPLC analysis.

HPLC Analysis:

The HPLC system consisted of a LC-20AT pump, SPD-20A UV, SIL-20AC auto injector, and CR3A integrator (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). The column used was a ZORBAX-SB-C18, 5µm 4.6 x 150mm (Agilent Technologies, Inc., Santa Clara, CA) and Aquapore ODS guard column, 7 µm, RP-18 (Thomson Instrument Company, Clear Brook, VA). The HPLC conditions were 50 µL injection volume, water-ACN gradient (25% ACN from 0-5 min, linear increase to 80% ACN from 5-15 minutes, and linear decrease to 25% ACN from 15-17 minutes, column regeneration time between injections of 8 minutes), UV detection at λmax 227nm, flow rate of 1.0 mL/minute, column temperature of 30°C. DTX and PTX elution times were 14.73 and 15.03 minutes, respectively. Peak area ratio was used to calculate DTX and PTX concentrations from a standard calibration curve obtained using plasma matrix standards.

Abraxane/Azaya Nanoliposome Sample Preparation:

Abraxane and Azaya nanolipsome samples were diluted to 25 µg/mL PTX or DTX, respectively, in 25% rat plasma-2% BSA.

DTX and PTX were added at a concentration of 3.13 µg/mL as internal standards for Abraxane and the Azaya nanoliposome samples, respectively. Samples were prepared in duplicate and immediately placed on ice prior to extraction.

Dynamic Light Scattering:

A Malvern Zetasizer Nano ZS instrument (Southborough, MA) with back scattering detector (173°) was used for measuring the hydrodynamic size (diameter) in batch mode at 25°C in a low volume quartz cuvette. The size was measured on a sample of the particle before tip extraction along with a sample from the tip flow through. The samples were prepared in saline at a concentration of 25 µg/ml. Data acquisition is repeated 3 times for each sample and the average is shown here.

Bradford Protein Assay:

The concentration of protein in the tip flow through and the pre-tip sample were determined using the quick start, Bradford protein assay kit 1, (Bio Rad, catalog # 500-0201). The Abraxane samples were prepared in saline at 25 µg/mL PTX.

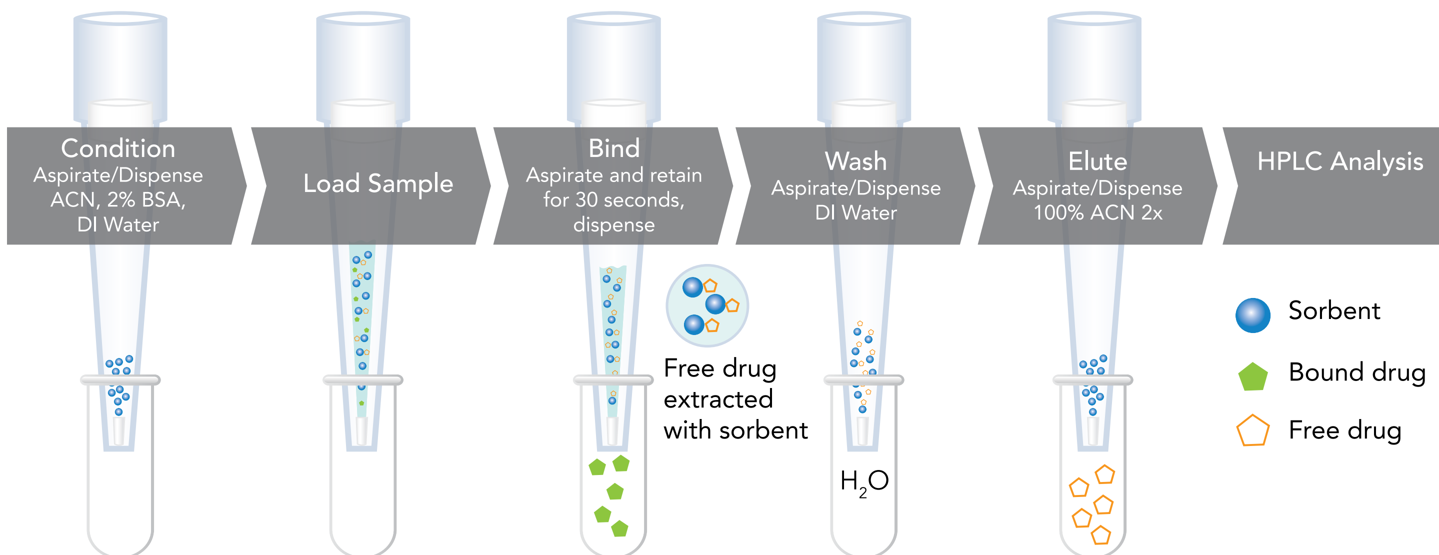

Figure 2. Dispersive Pipette XTRaction using RP-XTR tips.

RESULTS

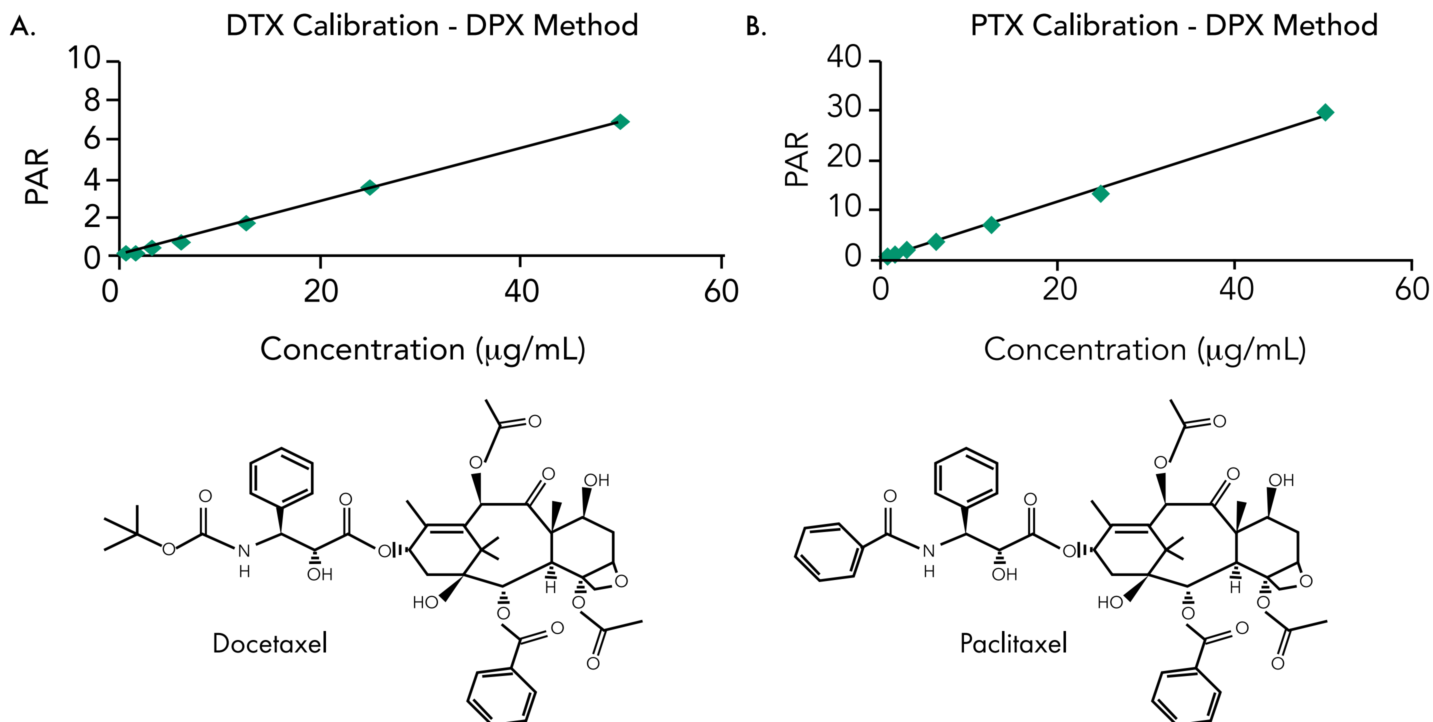

Figure 3. The DTX and PTX standard curves prepared in 25% rat plasma were extracted using 1 mL RP-XTR tips and quantified by HPLC analysis. The curves are presented as the mean of duplicate standards at each concentration, with peak area ratio (PAR) of DTX/ PTX area for the DTX curve (A), and PAR of PTX/ DTX area for the PTX curve (B). The extraction efficiency for DTX and PTX from plasma were consistently 70%.

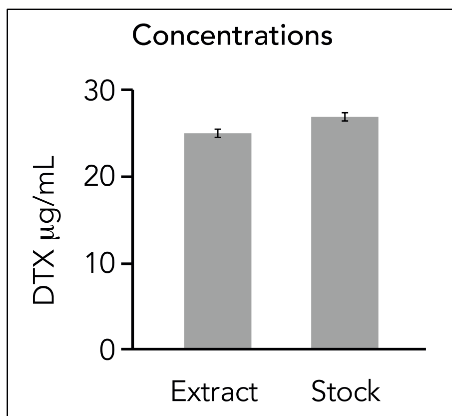

Figure 4. DTX concentrations in the original Azaya nanoliposome plasma stock solution, and RP-XTR extract are displayed. DTX is readily extracted from the Azaya nanoliposome in plasma. Data is presented as the mean±SD (n=2).

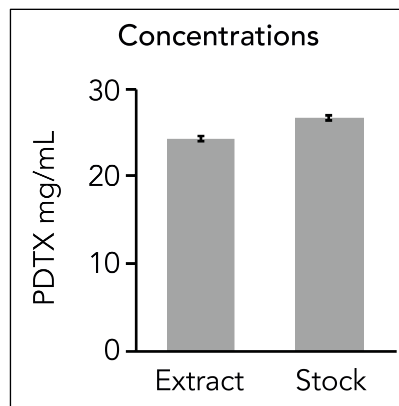

Figure 7. DPTX concentrations in the original Abraxane plasma stock solution, and RP-XTR extract are displayed. PTX is readily extracted from Abraxane in plasma. Data is presented as the mean±SD (n=2).

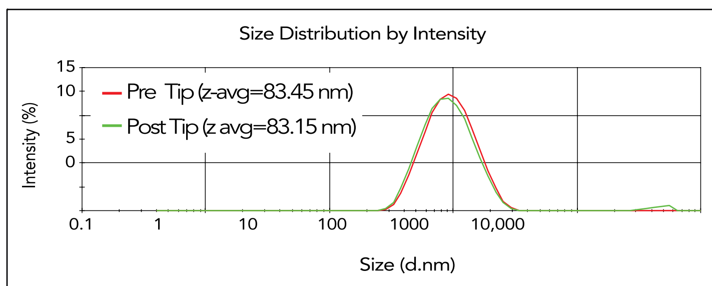

Figure 5. Particle size was measured by dynamic light scattering on pre and post tip saline samples (25 µg/mL DTX). There was minimal disruption of the particle from the extraction procedure. Data is presented as the mean (n=3).

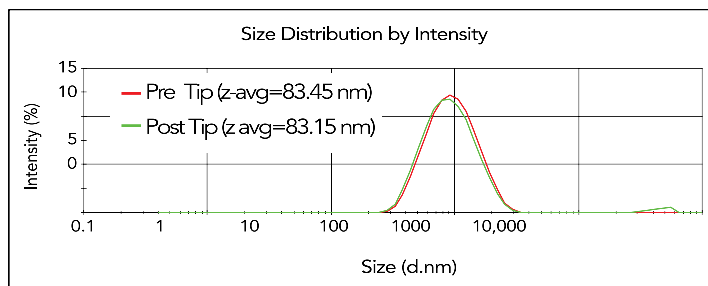

Figure 8. Particle size was measured by dynamic light scattering on pre and post tip saline samples (25 µg/mL PTX). The particle is disrupted by the extraction procedure. Data is presented as the mean (n=3).

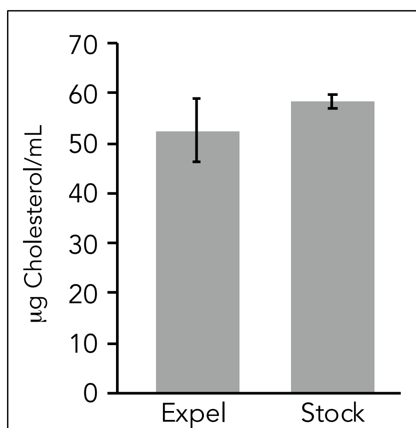

Figure 6. Cholesterol concentration was measured by HPLC in the pre-tip Azaya nanoliposome plasma stock and RP-XTR tip flow through. The majority of cholesterol was recovered from the post tip sample, suggesting the nanoliposome was not extracted. Endogenous cholesterol did not interfere with cholesterol measurement. This data represents the mean ± SD, N=2.

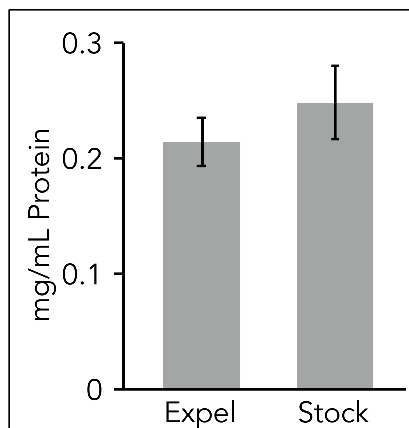

Figure 9. Protein concentration was measured in the pre-tip Abraxane saline stock and RP-XTR tip flow through. The majority of protein was recovered from the post tip sample, suggesting the albumin nanoparticle was not extracted. The BSA block did not interfere with protein measurement. This data represents the mean ± SD, N=2.

CONCLUSIONS

- Using RP - XTR tips, the extraction method efficiency was 70% for DTX and PTX in 25% rat plasma.
- The analyte in both formulations were readily extracted from the nanoformulations in biological matrix, this is consistent with existing pharmacokinetic data (1). By contrast, minimal amounts of the actual nanoparticles were extracted by this method.
- Dynamic light scattering showed that the nanoliposome was unaffected by the extraction procedure, while the albumin nanoparticle was disrupted.
- These data support the further investigation of this method for extraction of free drug from biological matrix, and to support the use of this method for estimation of drug release from a controlled release nanoformulation and determine what drugs and nanoparticle types will be applicable.