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

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Abstract

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 bioavailable, free drug profile in pharmacokinetic samples. These studies could aid in the design of nanoformualtions with better controlled release properties, as well as aid in safety assessment and estimation of a clinical starting dose. The present study utilizes a particulate, solid phase extraction method for 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 (1), 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 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. Further study is needed 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.

Experimental Procedure

Materials

DPX Reverse Phase Tips, 1mL (Catalog #RP-1) were purchased from DPX Labs, LLC (Columbia, SC). Borosilicate glass tubes, 12.75mm (Catalog # 14-961-26) were purchased from Fisher Scientific (Pittsburgh, PA). BSA (Catalog # A4503-100G) and Acetonitrile, gradient grade for HPLC (Catalog # 439134-1L) were purchased from Sigma (St. Lous, MO). Rat plasma (Catalog # INR-N12) was purchased from Innovative Research (Novi, Michigan). Paclitaxel (PTX) (Catalog # P-9600) and docetaxel (DTX) (Catalog # D-100) were purchased from LC Laboratories (Woburn, MA). HPLC sample 96 well V-bottom plate (Catalong # 6008290) were purchased from Perkin Elmer (Waltham, MA). Silicone sealing mat (Catalog # AM-2ML-RD) were purchased from Axygen (union City, CA).

RP-DPX Tip Preparation

The tips were first activated by drawing 250µL of acetonitrile, drawing air to mix and then dispensing to waste, then repeated using DI water. The tips were then blocked to prevent nonspecific extraction of the nanoparticle by drawing 250µL of 2% BSA, drawing air to mix, then dispensing to waste. The blocking step was repeated once more with 2% BSA, and finally washed with DI water.

RP-DPX Extraction Method

250µl of the sample was drawn into the blocked tip, air was drawn to mix sorbent, the sample was retained for 30 sec, and then dispensed to waste. Next, 250µl of DI water was drawn into the tip, air was drawn to mix, and then dispensed to waste. Next 250µl of 100% ACN was drawn into the tip, air was drawn to mix sorbent, and then dispensed to a collection tube. The ACN extraction was repeated, and the eluent dispensed to the same 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 min, and thawed and centrifuged for 20 min at 14kg. The supernatant was transferred to a glass borosilicate tube, and dried under nitrogen at 48C in a turbo VAP. The dried sample residues were reconstituted in 500uL of 50/50 ACN-DI water and analyzed by HPLC analysis.

DTX/PTX HPLC Analysis

The HPLC system consisted of a LC-20AT pump, SPD-20A UV, SIL-20AC auto injector, and C-R3A integrator (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). The column used was a ZORBAX-SB-C18, 5µm 4.6 x 150mm (Catalog # 883975-902, Agilent Technologies, Inc., Santa Clara, CA) and Aquapore ODS guard column, 7µm, RP-18 (Catalog # 0711-0092, Thomson Instrument Company, Clear Brook, VA). The HPLC conditions were 50µL injection volume, water-acetonitrile (ACN) gradient (25% ACN from 0-5min, linear increase to 80% ACN from 5-15 min, and linear decrease to 25% ACN from 15-17 min, column regeneration time between injections of 8 min), UV detection at λ_{max} 227nm, flow rate of 1.0 mL/min, column temperature of 30°C. DTX and PTX elution times were 14.73 and 15.03 min, 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 25ug PTX or DTX/mL, 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.

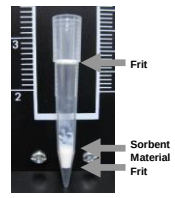
Dynamic Light Scattering

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 ug /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 PTX/ml.

A.



B.

Fig. 1. DPX Reverse Phase Tips
RP-DPX tips (A) and syringe mounting (B).

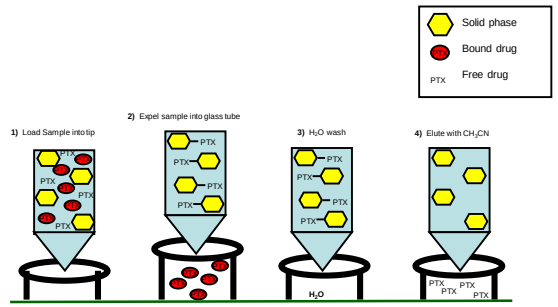
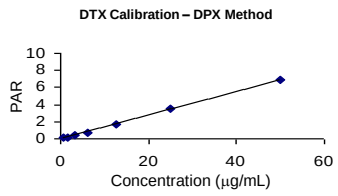
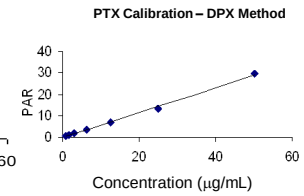


Fig. 2. RP-DPX Extraction Procedure

A.



B.



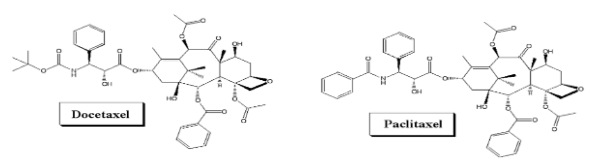


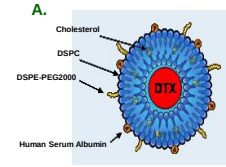
Fig.3. DTX/PTX Calibration Curves
The DTX and PTX standard curves prepared in 25% rat plasma were extracted by the RP-DPX method and quantified by HPLC analysis. The curves are presented as the mean of duplicate standards at each concentration, with peak area ratio (PAR) of DTX/ PTX area for the DTX 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%.

References

Randomized crossover pharmacokinetic study of solvent-based paclitaxel and nab-paclitaxel.Gardner ER, Dahut WL, Scripture CD, Jones J, Aragon-Ching JB, Desai N, Hawkins MJ, Sparreboom A, Figg WD. Clin. Cancer Res. 2008 Jul 1;14(13):4200-5.

RP-DPX Extraction: Azaya DTX Nanoliposome

A.



B.

AZAYA THERAPEUTICS

Fig. 4. Azaya Therapeutics Nanoliposome
The Azaya Nanoliposome is protein stabilized DTX liposomal fomulation, 83 nm in size. A schematic of the nanoliposme (**A**) and commercial logo (**B**) are displayed.

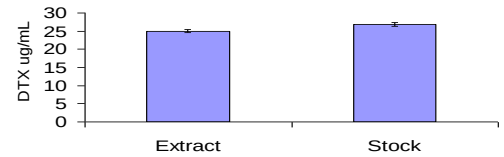


Fig. 5. Azaya Nanoliposme RP-DPX Plasma Extraction
DTX concentrations in the original Azaya nanoliposome plasma stock solution, and RP-DPX extract are displayed. DTX is readily extracted from the Azaya nanoliposome in plasma. Data is presented as the mean±SD (n=2).

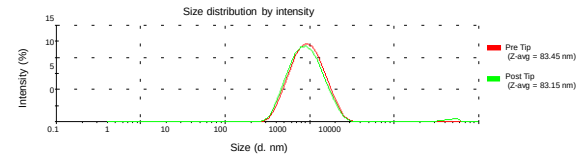


Fig. 6. Size Analysis- DLS
Particle size was measured by dynamic light scattering on pre and post tip saline samples (25 ug DTX/mL). There was minimal disruption of the particle from the extraction procedure. Data is presented as the mean (n=3).

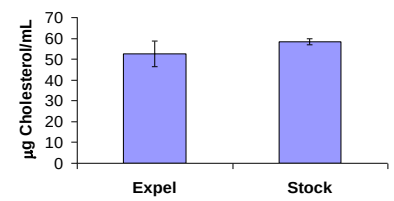


Fig. 7. Cholesterol Recovery
Cholesterol concentration was measured by HPLC in the pre-tip Azaya nanolipome plasma stock and RP-DPX tip flow through. The majority of cholesterol was recovered from the post tip sample, suggesting the nanoliposome was not extracted. Endogenous cholesterol did not interfere with cholesterol measurement. This data represents the mean ± SD, N=2.

Acknowledgements

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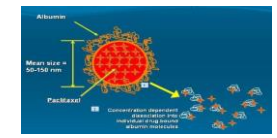
RP-DPX Extraction: Abraxane™ PTX Albumin Nanoparticle

A.



B.

Fig. 8. Azaya Therapeutics Nanoliposome
Abraxane is a albumin nanoparticle formulation of paclitaxel, 130nM in size. A schematic of Abraxane(**A**) and clinical dosage (**B**) are displayed.

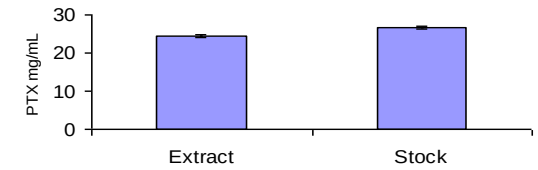


Fig. 9. Abraxane RP-DPX Plasma Extraction
DPTX concentrations in the original Abraxane plasma stock solution, and RP-DPX extract are displayed. PTX is readily extracted from Abraxane in plasma. Data is presented as the mean±SD (n=2).

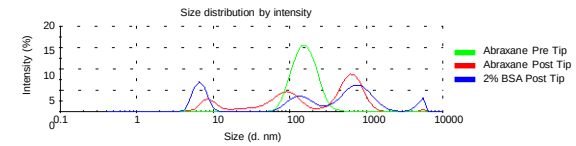


Fig. 10. Size Analysis- DLS
Particle size was measured by dynamic light scattering on pre and post tip saline samples (25 ug PTX/mL). The particle is disrupted by the extraction procedure. Data is presented as the mean (n=3).

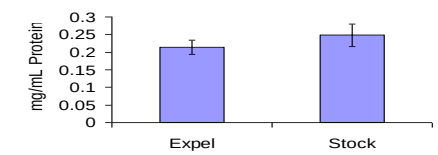


Fig. 3. Protein Recovery
Protein concentration was measured in the pre-tip Abraxane saline stock and RP-DPX tip flow through. The majority of protein was recovered from the post tip sample, suggesting the albumin nanoparticle was not extracted. The BSA block did not interfer with protein measurement. This data represents the mean ± SD, N=2.

Conclusion

- This RP-DPX method extraction 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.

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