

# Quantitative Determination of Paclitaxel in Human Whole Blood (HWB) Dried Blood Spots (DBS) using LC/MS/MS

Khanh Nguyen, Dale Schoener, Michael Buonarati  
Alta Analytical Laboratory, El Dorado Hills, CA

## Purpose

To develop and validate an LC/MS/MS method to quantify paclitaxel in human whole blood (HWB) dried blood spots (DBS) and to compare the DBS method to an existing conventional SPE method.

Figure 1. Structure of Paclitaxel

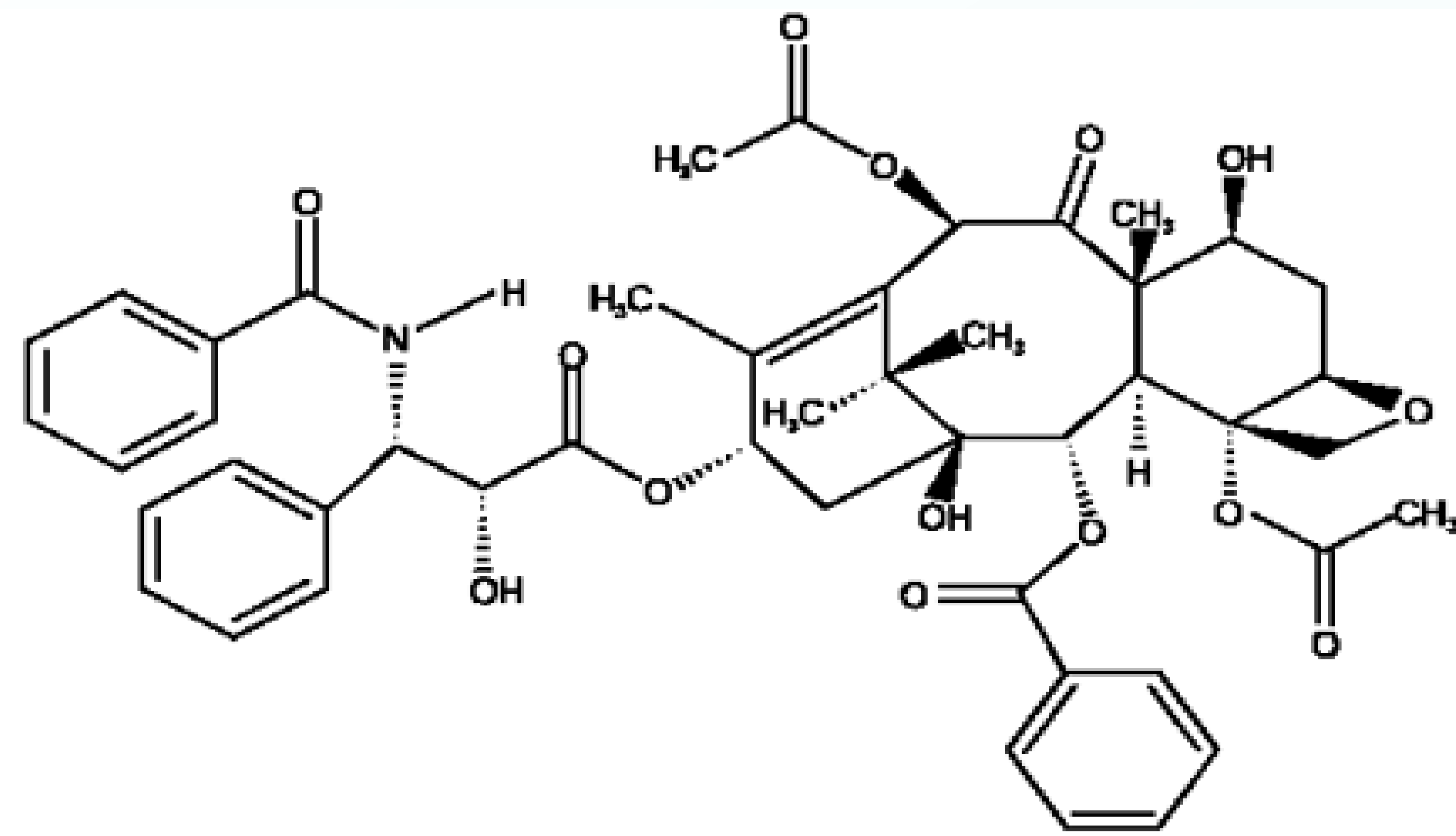
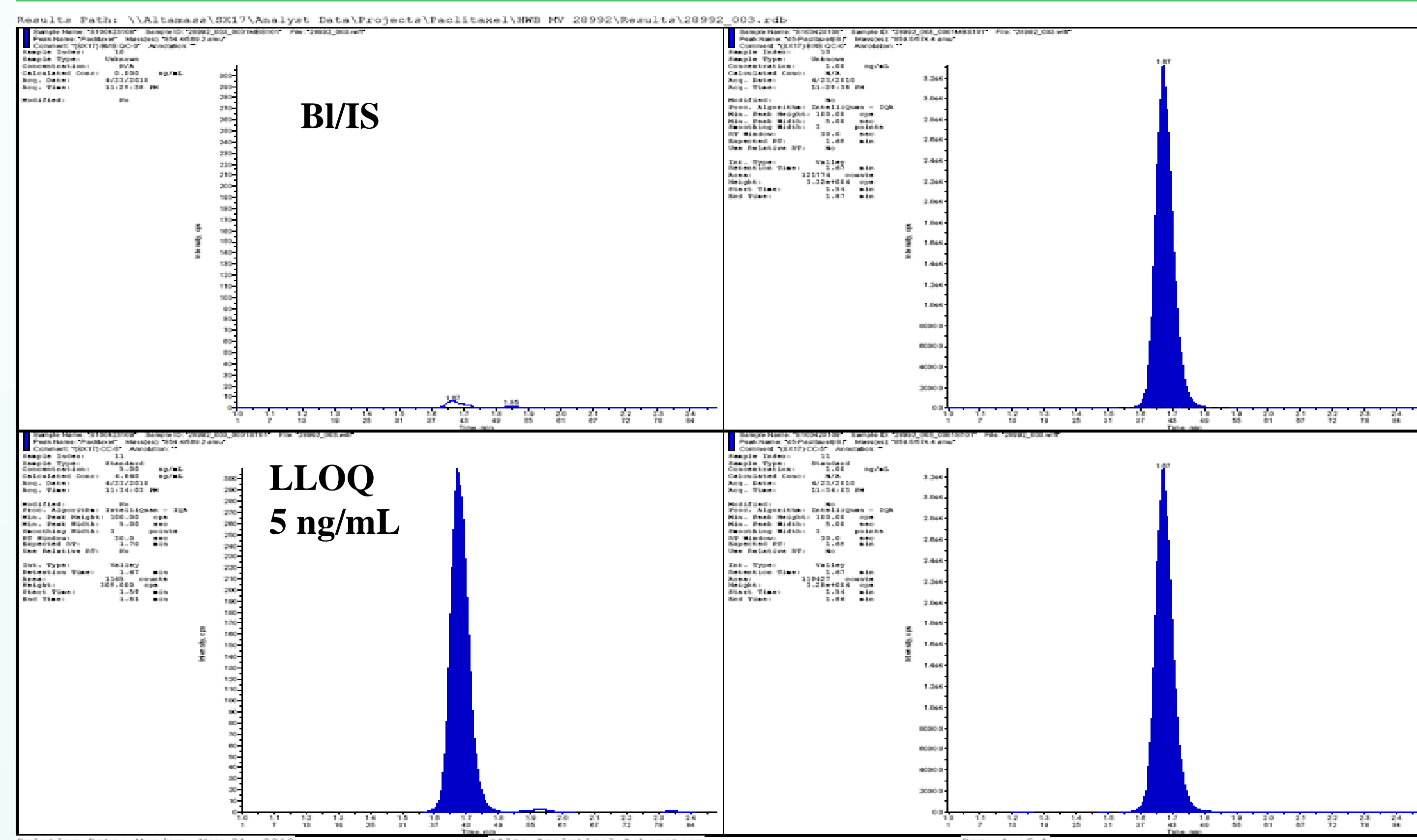


Figure 2. Chromatograms from DBS (2.3 µL sample aliquot with a 3 mm diameter punch)



## Method

### DBS Extraction

- 15 µL of HWB spotted onto Whatman FTA® DMPK-C cards.
- Allow to dry for at least 3 hours
- 3 mm diameter punch is made from DBS
- Paclitaxel is extracted from HWB DBS using MeOH as the extraction solvent. The internal standard is d<sub>5</sub>-Paclitaxel.
- Supernatant is further diluted 1:1 with 0.1% FA.

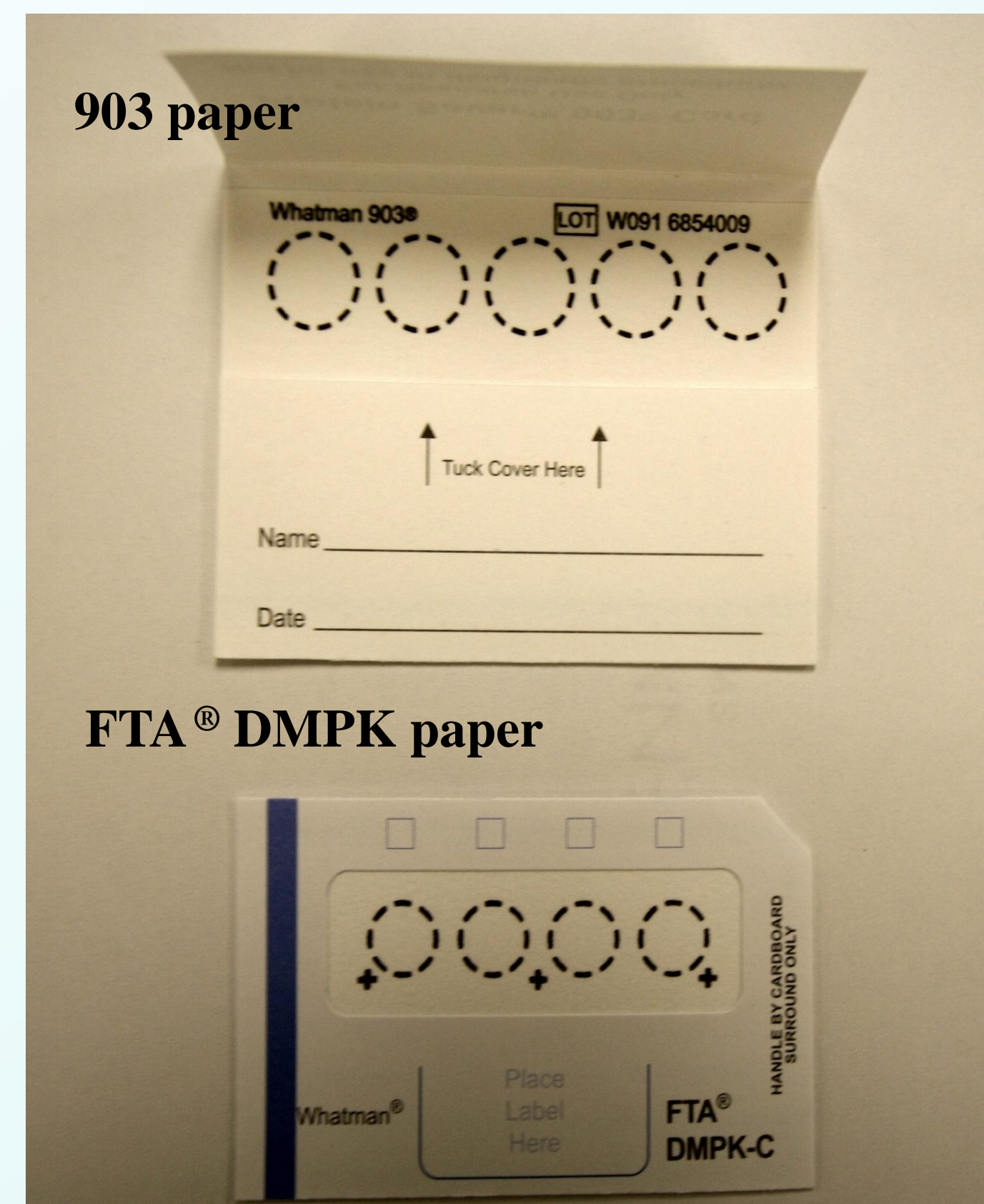
### LC/MS/MS

- MPA: 5 mM NH<sub>4</sub>HCO<sub>3</sub>
- MPB: MeOH with 5 mM NH<sub>4</sub>HCO<sub>3</sub>
- ACE® 3 Phenyl, 2.1 x100 mm, 3 µm at 50°C
- 20 µL of final extract injected onto column
- 0.7 mL/min
- API-5000, positive TIS, m/z 854.4 – 569.2; IS (d<sub>5</sub>-paclitaxel) 859.5 – 874.4
- 0.00 min 60%B, 0.50 min 60%B, 0.51 in 70%B, 2.0 min 75%B
- Run time is approximately 4 minutes, RT 1.7 min (k' ~ 4)

### Blood Spotting Procedure

- For FTA® DMPK-C cards, 15 µL is the recommended volume.
- Expel a few mm above the card. Card should not be touched.
- Dry 2-3 hours.
- Sample from the middle of the blood spot. 3 mm punch recommended.
- Store ambient temperature desiccated.

### Figure 3. Paper Options



### Paper Choice Based on Reproducibility

	903 paper	FTA®-DMPK paper
CV = 5.9%		CV = 3.2%
n = 8		n = 8
Residual error: replicates of a pooled final extract: CV ~ 1.3%		

### DBS vs. conventional validation

- Essentially the same as a conventional validation.
  - Punch carryover
  - Still do AQL dilution, although trickier
  - 24 RT BT plasma or WB stability; now 7 day RT BT, no desiccant, exposed to light
  - Punch qualification instead of pipette qualification
- Not needed anymore.
  - Freeze thaw stability
  - No whole blood stability

Table 1. Assessment of Center punch vs. Perimeter punch

	mean	SD	CV	n
Center punch	0.788	0.0180	2.3	n=6
Perimeter punch	0.808	0.0178	2.2	n=6

### DBS AQL Dilutions

- Fundamental difference in how dilutions are performed.
- With conventional methods the WB or plasma sample is diluted with WB or plasma matrix and then extracted.
- With DBS methodology the sample is extracted first.
  - Several blank matrix DBS are extracted. The extraction solvents and IS are added to these samples. This is performed scaled up in a single tube where the same punch/solvent/IS ratio is maintained.
  - The extracted sample is then diluted with this Blank/IS final extract.

### Accuracy Assessment : Comparing concentration obtained to spiked in concentration to and comparison of DBS method to conventional HWB SPE Method

- Experimental Design
  - Spike 15 individual female and 15 individual male HWB samples between 6 ng/mL and 900 ng/mL.
  - Each sample spiked at a unique concentration between 6 and 900 ng/mL.
  - Assay each spike in duplicate, (total 30 samples), 60 determinations by the DBS method and 60 determinations by the conventional HWB SPE method.
- Objectives
  - DBS accuracy determined by comparing concentration obtained to nominal concentration.
  - Compare concentration of DBS method to conventional HWB method.

Table 4. DBS Female

Nominal Concentration (ng/mL)	Accuracy (%) n=2		HCT
6	106	101	38
7	98	99	43
9	97	102	42
20	109	104	42
40	106	96	42
70	108	93	38
90	98	101	38
125	101	94	38
200	100	95	39
300	99	99	41
400	96	100	44
600	92	92	38
700	103	103	41
850	105	100	41
900	95	97	38

Table 5. DBS Male

Nominal Concentration (ng/mL)	Accuracy (%) n=2		HCT
6	100	106	48
7	96	102	41
9	111	97	38
20	110	103	41
40	99	91	43
70	95	96	40
90	103	99	38
125	103	99	40
200	106	106	39
300	105	108	41
400	107	103	43
600	107	99	38
700	101	99	43
850	100	103	41
900	98	99	39

Table 6. Precision comparison of DBS vs Conventional SPE Method

	DBS	HWB SPE conventional
Within-run error (CV) 6 and 7 ng/mL levels	3.4	0.7
Within-run error (CV) 300 and 400 ng/mL levels	2.5	1.2

Table 2. Assessment of Variable Hematocrit

	mean	SD	CV	n
50% HCT	0.809	0.0232	2.9	n=6
25% HCT	0.722	0.0127	1.8	n=6

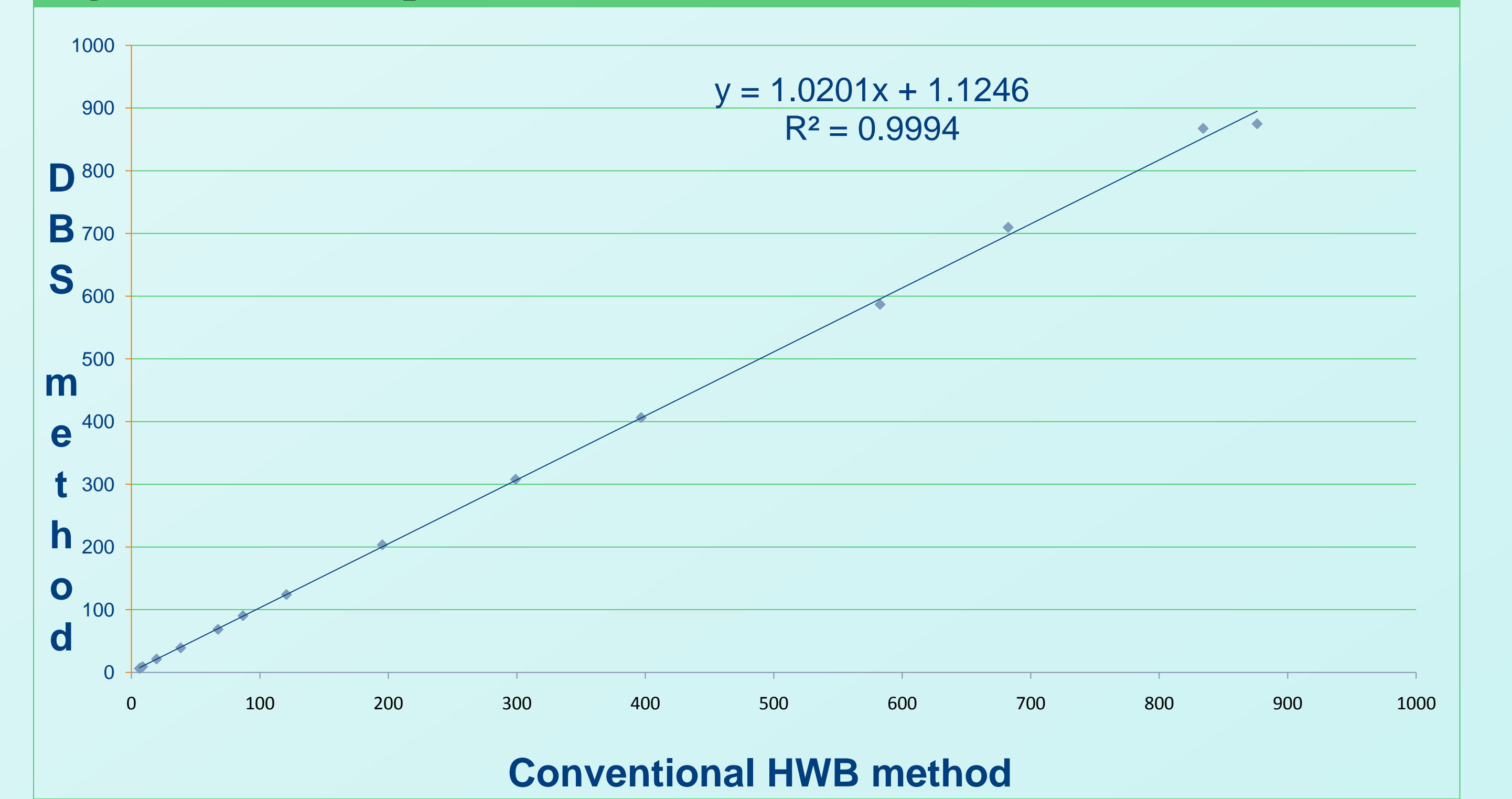
Table 3. Assessment of Vortex Mixing vs. Sonication

	mean	SD	CV	n
Vortex 5 minutes	0.776	0.0334	4.3	n=4
Vortex 10 minutes	0.776	0.0093	1.2	n=4
Vortex 15 minutes	0.778	0.0306	3.9	n=4
Sonicate 4 minutes	0.794	0.0269	3.4	n=4

Table 7. DBS vs. Conventional HWB SPE Method Individual spikes

Nominal Concentration (ng/mL)	Mean Concentrations (ng/mL) n=4 (combined male and female)		% difference between DBS and HWB
	DBS	HWB conventional	
6	6.19	6.25	-0.9
7	6.92	6.80	1.8
9	9.17	8.70	5.2
20	21.3	19.5	8.6
40	39.2	38.3	2.1
70	68.7	67.4	1.9
90	90.4	86.8	4.1
125	124	121	2.9
200	203	195	4.1
300	308	299	2.9
400	406	397	2.4
600	587	583	0.7
700	710	682	3.9
850	867	834	3.9
900	875	876	-0.2

Figure 4. The Comparison of DBS vs. Conventional HWB SPE Method



## Results and Discussion

Paclitaxel was validated successfully in DBS over the 5 to 1000 ng/mL range. Signal to noise at the LLOQ was greater than 100:1. Recovery assessed at the low, mid and high quality control (QC) levels averaged approximately 106%. Inter-assay % deviation values ranged from 0.750% to 3.42%. Inter-assay precision (%CV) values ranged from 3.63% to 5.62%. The accuracies in individual male and female samples for the DBS method ranged from 93% to 107% (Tables 4 and 5).

Assessment of a center vs. perimeter punch of a DBS show s only a 2.5% difference. (Table 1). Hematocrit affects the size and dispersal of a blood spot. The difference in recovery between a sample with 50% and 25% hematocrit level was 10% (normal hematocrit is mid 30% to mid 40%), see Table 2. Assessment of vortex mixing vs. sonication (Table 3) shows that recovery does not significantly change with increased vortex mixing time over 5 minutes or with the use of sonication in place of vortex mixing.

Agreement between the DBS and conventional SPE method was assessed by spiking 30 individual lots (15 female and 15 male) of HWB over a 6 to 900 ng/mL range. The same calibration curve and QC sample preparations were used for both DBS and conventional SPE methods. Comparison of the mean concentrations of the individual spikes of DBS to the SPE method showed a percent difference range of -1% to 8.6% (Table 7 and Figure 4).

## Conclusion

The validated method for the quantification of Paclitaxel in HWB DBS is simple, sensitive and accurate. The results of the DBS method match very closely to the existing conventional SPE HWB method assessed over the 5 to 1000 ng/mL range.

## References

- Edelbroek, Peter M. and et al., "Dried Blood Spot Method in Therapeutic Drug Monitoring: Methods, Assays, and Pitfalls." *Therapeutic Drug Monitoring* 2009, vol 31: 327-336.
- Spooner, Neil and et al., "Dried Blood Spots as a Sample Collection Technique for the Determination of Pharmacokinetics in Clinical Studies: Considerations for the Validation of a Quantitative Bioanalytical Method." *Analytical Chemistry* 2009, vol 81: 1557-1563
- Spooner, Neil, "The Use of Dried Blood Spot Samples for the Quantitative Bioanalysis of Drugs in Preclinical & Clinical Studies." *PCD DMPK, GlaxoSmithKline, Ware, UK* 2009.
- Emmons, G., *The Future of Dried Blood Spots in Drug Development*, AAPS, 2009