

Supplementary Data

A Promising Microtubule Inhibitor Deoxypodophyllotoxin Exhibits Better Efficacy to Multi-Drug Resistant Breast Cancer than Paclitaxel via Avoiding Efflux Transport

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Drug metabolism and disposition

1. Linearity

The standard curve exhibited a good linear relationship over the concentration range of 0.5-1000 ng/mL with a regression coefficient ($r^2=0.9992 \pm 0.0003$).

2. Recovery and matrix effect

Across the 5 independent experiments, the mean recovery of DPT ranged from 94.13% to 104.76% over the three concentrations. The precision was < 5.59%, which proved the reproducibility and consistency of the extraction method. Due to the matrix effect ranging from 98.27% to 102.29% and the precision < 5.85%, the suppression or enhancement could be neglected under the current conditions.

3. Precision and accuracy

The intra- and inter-day precision and accuracy values for 5 replicates of QC samples at three nominated concentrations were shown in Table S1, which strongly supported the accuracy, reliability and reproducibility of the method.

Table S1 Intra- and inter-day precision and accuracy for DPT (n=5)

Nominal concentration (ng/ml)	Intra-day			Inter-day		
	Measured concentration (mean±SD, ng/ml)	Accuracy (RE, %)	Precision (RSD, %)	Measured concentration (mean±SD, ng/ml)	Accuracy (RE, %)	Precision (RSD, %)
1	1.00±0.06	0.00	6.00	1.00±0.09	0.01	8.60
20	21.14±0.69	5.70	3.26	20.87±0.70	4.37	3.34
1000	963.80±20.14	-3.62	2.09	960.93±28.75	-3.91	2.99

4. Stability

The stability of DPT was investigated under different conditions and was shown in Table S2, thus indicating that the samples were all stable under three freeze-thaw cycles, at -80 °C for 14 days and at room temperature for 24 h and under post-preparation in an autosampler tray (4 °C) for 24 h.

Table S2 Stability of DPT under different conditions at three QC levels (n=5)

Storage conditions	Nominal concentration (ng/ml)	Measured concentration (mean±SD)	Accuracy (RE, %)	Precision (RSD, %)
Three freeze-thaw cycles	1	0.98±0.06	-2.22	6.32
	20	20.40±0.93	2	4.55
	1000	983.60±18.83	-1.64	1.91
Frozen (-80°C) for 14 days	1	0.85±0.08	-14.96	9.42
	20	20.20±0.71	1	3.53
	1000	961.80±39.76	-3.82	4.13
Room temperature for 24h	1	1.00±0.06	0.26	5.72
	20	21.18±0.32	5.9	1.51
	1000	963.40±45.91	-3.66	4.77
Post-preparation (4 °C) for 24h	1	1.03±0.10	2.56	9.72
	20	20.68±0.51	3.4	2.48
	1000	946.40±35.51	-5.36	3.75

5. Recovery and matrix effect

Table S3 Recovery and matrix effect of DPT in mitochondria (n=5)

Analytes	Spiked concentration (ng/mL)	Recovery (mean±SD%)	RSD(%)	Matrix effect (mean±SD%)	RSD(%)
DPT	1.00	100.86%±1.39%	1.38%	106.19%±3.16%	2.98%
	20.00	104.20%±5.06%	4.85%	98.74%±6.49%	6.57%
	1000.00	99.66%±5.90%	5.92%	97.08%±3.26%	3.36%

Table S4 Recovery and matrix effect of DPT in nuclei (n=5)

Analytes	Spiked concentration (ng/mL)	Recovery (mean±SD%)	RSD(%)	Matrix effect (mean±SD%)	RSD(%)
DPT	1.00	97.67%±7.13%	7.30%	106.16%±4.92%	4.63%
	20.00	103.91%±4.52%	4.35%	94.25%±6.53%	6.93%
	1000.00	99.85%±2.45%	2.45%	94.85%±4.46%	4.71%

Table S5 Recovery and matrix effect of DPT in cytosol (n=5)

Analytes	Spiked concentration (ng/mL)	Recovery (mean±SD%)	RSD(%)	Matrix effect (mean±SD%)	RSD(%)
DPT	1.00	95.13%±1.50%	1.57%	110.73%±3.65%	3.30%
	20.00	101.31%±5.69%	5.61%	101.64%±4.71%	4.64%
	1000.00	102.79%±7.02%	6.83%	106.80%±4.72%	4.42%

Table S6 Recovery and matrix effect of DPT in blood (n=5)

Analytes	Spiked concentration (ng/mL)	Recovery (mean±SD%)	RSD(%)	Matrix effect (mean±SD%)	RSD(%)
DPT	15.60	102.70%±5.17%	5.03	92.55%±4.92%	5.32
	125.00	107.72±4.68%	4.35	97.83%±7.21%	7.36
	500.00	96.75%±5.61%	5.80	98.88%±6.75%	6.82

Table S7 Recovery and matrix effect of DPT in liver (n=5)

Analytes	Spiked concentration (ng/mL)	Recovery (mean±SD%)	RSD(%)	Matrix effect (mean±SD%)	RSD(%)
DPT	15.47	100.54%±3.05%	3.54	97.22%±5.01%	5.15
	123.75	91.98%±2.95%	3.21	82.65±0.78%	0.94
	792.00	93.48%±3.42%	3.65	100.43%±2.74%	2.73

6. Quality control

Table S8 Quality control of DPT in heart, spleen, lung, kidney and tumor (n=5)

Analytes	Spiked concentration (ng/mL)	Heart		Spleen		Lung		Kidney		Tumor	
		Measured concentration (mean±SD%)	RE(%)	Measured concentration (mean±SD%)	RE(%)	Measured concentration (mean±SD%)	RE(%)	Measured concentration (mean±SD%)	RE(%)	Measured concentration (mean±SD%)	RE(%)
DPT	15.47	16.75±0.09	8.27	16.68±1.01	7.79	16.41±0.33	6.08	15.95±0.83	3.10	14.33±0.30	-7.37
	123.75	127.89±11.65	3.34	119.02±4.74	-3.82	131.04±6.25	5.89	127.65±4.99	3.15	130.36±3.55	5.34
	792.00	737.87±8.74	-6.83	778.74±0.28	-1.67	777.30±6.31	-1.86	1749.63±18.04	-5.35	746.87±10.49	-5.70