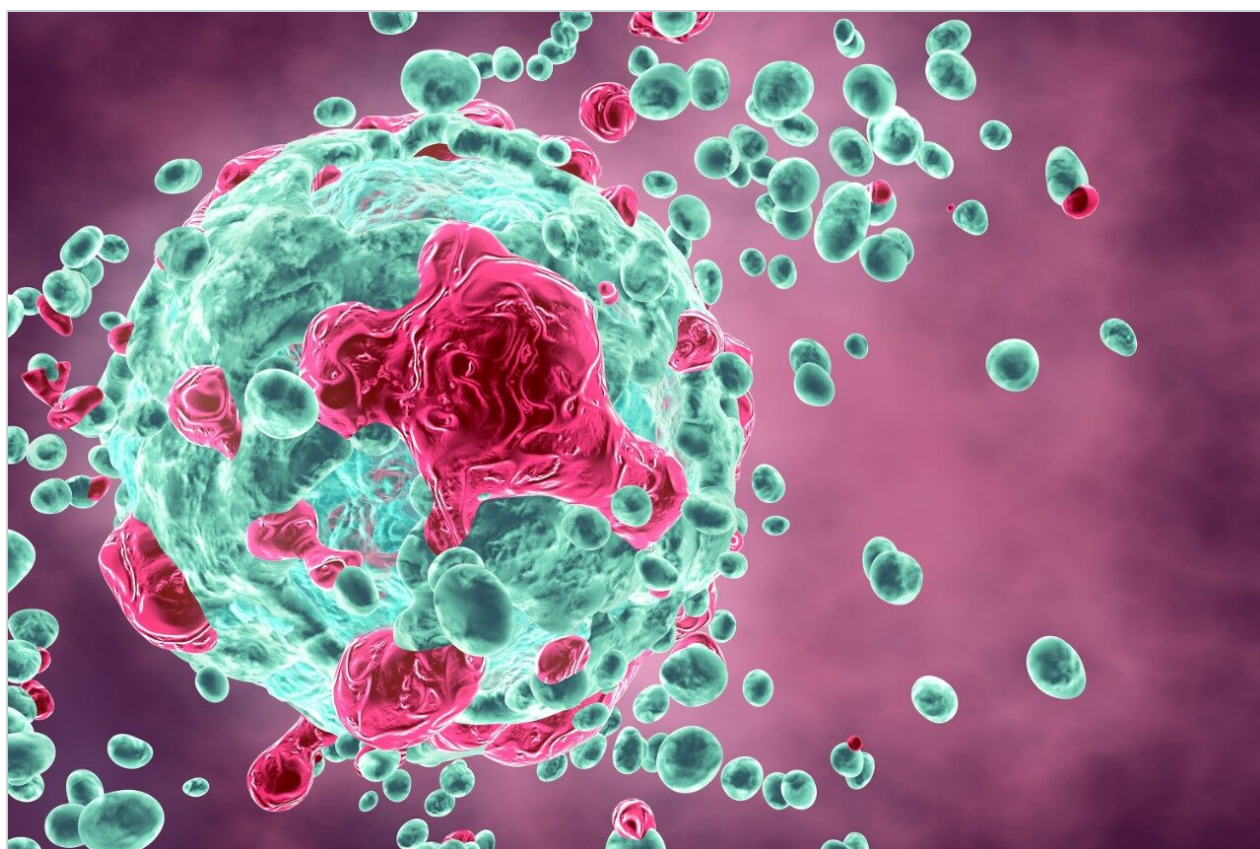# Waters™

A Novel Extraction Procedure Using Micro Elution Plates for the Estimation of Docetaxel Using UPLC and ACQUITY TQD

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Abstract

This application note focuses on the use and benefits of micro-elution extraction methods and also the use of the ACQUITY TQ Detector in obtaining a signal-to-noise (S/N) ratio of ~225 at the LLOQ level for docetaxel.

Benefits

- Demonstrates the benefits of ACQUITY TQD for monitoring multiple transitions in compounds

- Oasis Micro-elution Plates ensure high-throughput sample extraction of compounds

- Addresses several key challenges faced by today's bioanalytical scientist in acquiring fast, high, accurate, and robust LC-MS results

- Maintains high throughput capability.

Introduction

Docetaxel is a clinically well-established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer. It belongs to the chemotherapy drug class of "Taxane", and is a semi-synthetic analogue of Paclitaxel (Taxol). Docetaxel binds to microtubules reversibly with high affinity and has a maximum stoichiometry of 1 mole docetaxel per mole tubulin in microtubules.
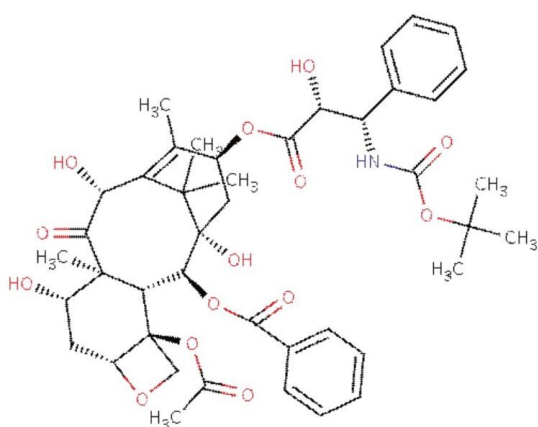

Figure 1. Molecular structure of docetaxel.

This molecule also has high protein binding nature, and it has been reported that protein disruption results in improper assay results. Hence, it is highly challenging to extract such types of molecules. Micro-elution offers the advantage of using less plasma volume, coupled with the fact that the sample cleanup can be done effectively. This application note focuses on the use and benefits of micro-elution extraction methods and also the use of the ACQUITY TQ Detector in obtaining a signal-to-noise (S/N) ratio of ~225 at the LLOQ level for docetaxel.

Experimental

LC conditions

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LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_8 1.7-µm, 2.1 x 100 mm
LC column elution:	70% aqueous buffer over 2.0 min followed by a 90% organic elution until 4.2 min; then change back to initial conditions.
Column temp.:	40 °C
Flow rate:	0.300 mL/min
Injection volume:	20 µL

MS conditions

MS system:	ACQUITY TQD
MS mode:	ESI positive MS/MS method
MRM transition:	808.7 $\rightarrow$ 226.3 and 808.7 $\rightarrow$ 282.3

The analyte from the spiked plasma samples was isolated using solid phase micro-elution extraction employing Waters Oasis HLB Micro-Elution Plates. A 300-µL aliquot of plasma was diluted with water, centrifuged at 12,000 rpm in a micro-centrifuge, and loaded onto SPE cartridges previously conditioned with organic solvent and water. The SPE cartridges were then washed with water twice followed by an organo-aqueous wash, and the samples were then eluted with the elution solvent. The eluted samples were injected on to the system directly.

Results and Discussion

Docetaxel eluted with a retention time of 2.67 mins and with a peak width of 10 s at the base. The data shown below illustrates the blank signal, shown in Figure 3, as well as the signal obtained from the lower limit of quantification (LLOQ) of docetaxel in human plasma. It can be observed that the analyte was well resolved from co-eluting peaks coming from the endogenous plasma components, shown in Figure 2. Figure 2 also shows chromatograms of six LLOQ samples and their respective signal-to-noise (S/N) ratios. The average of the S/N ratios was found to be 196.33.
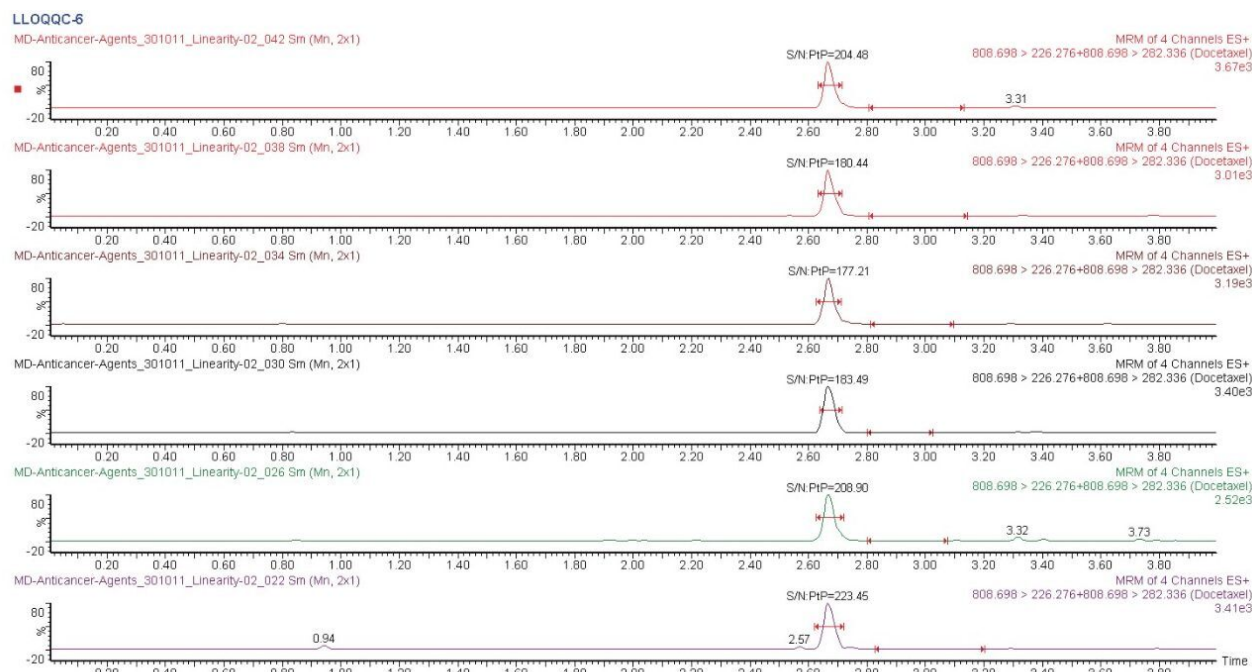
Signal-to-noise ratio of six replicates of docetaxel in LLOQ						
LLOQ1	LLOQ2	LLOQ3	LLOQ4	LLOQ5	LLOQ6	AVERAGE
204.48	180.44	177.21	183.49	208.9	223.45	196.33

Figure 2. Chromatogram of docetaxel at the LLOQ concentration of 200 pg/mL, along with the S/N ratios and the average S/N ratio obtained for LLOQs of six samples.
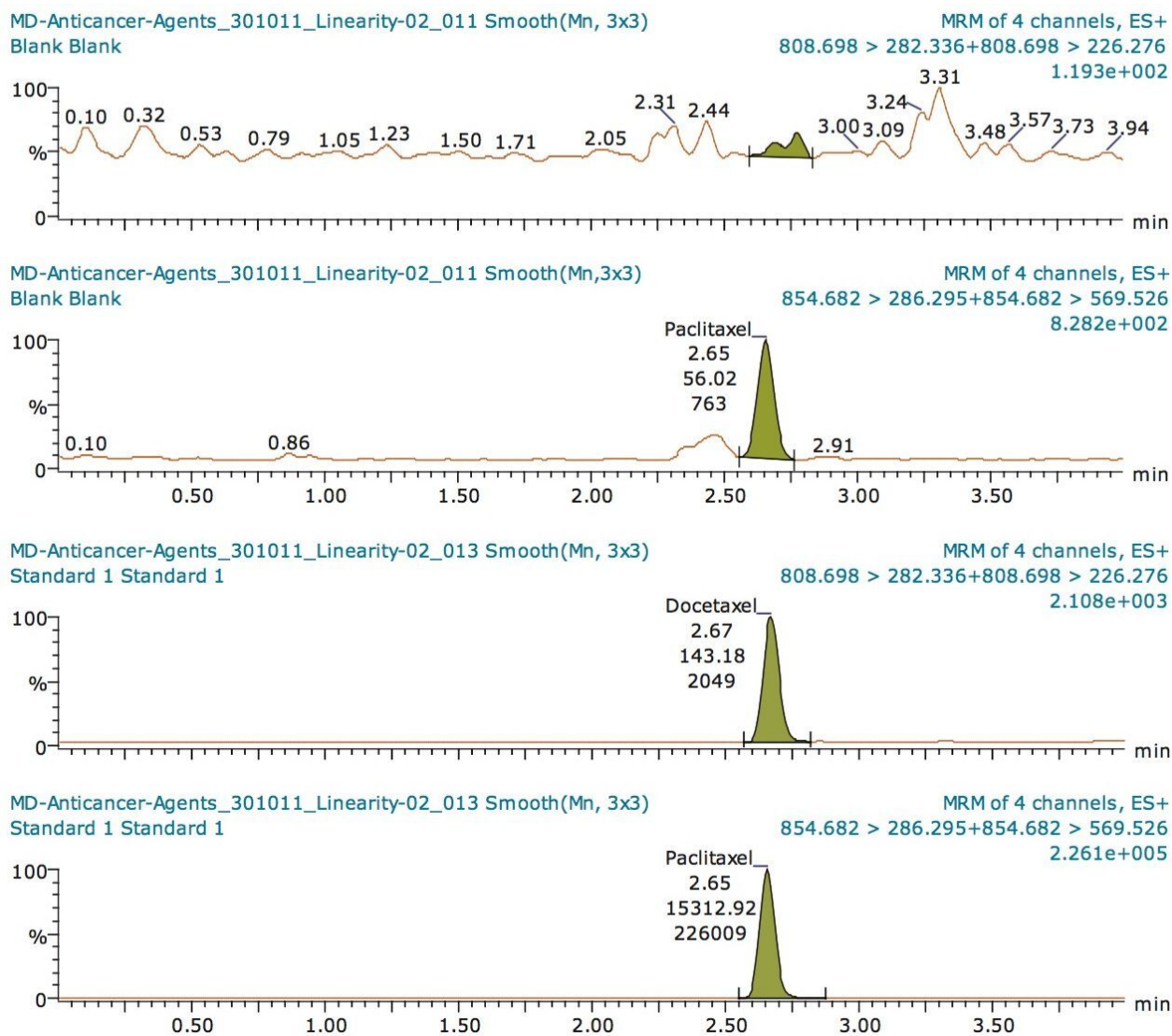
Figure 3. Chromatogram of blank and LLOQ concentration (200 pg/mL) of docetaxel.

The assay in this report showed a linear calibration over the range of 200 pg/mL to 100 ng/mL with an excellent r^2 value of 0.9994, shown in Table 1 and Figures 3.1 and 3.2. The back-calculated concentration of the standard was found to be within 12% of the nominal concentration, shown in Table 1.

Sample	Type	Nominal (ng/mL)	Analyte Area	IS Area	Area Ratio	Calculated (ng/mL)	Accuracy
Blank	Blank	Blank	2	56			
Standard 1	Standard	0.2	2.67	143	15313	0.00935	99.38
Standard 2	Standard	0.6	2.67	431	15789	0.02730	101.39
Standard 3	Standard	1.0	2.67	742	16665	0.04454	100.20
Standard 4	Standard	2.0	2.67	1411	15867	0.08895	100.78
Standard 5	Standard	5.0	2.67	3444	15509	0.22206	101.08
Standard 6	Standard	10.0	2.67	6798	15280	0.44491	101.40
Standard 7	Standard	20.0	2.67	13289	15721	0.84532	96.40
Standard 8	Standard	50.0	2.67	33431	14920	2.24076	102.26
Standard 9	Standard	100.0	2.67	61645	14488	4.25499	97.11

Table 1. Calibration data of docetaxel over the range of 200 pg/mL to 100 ng/mL.
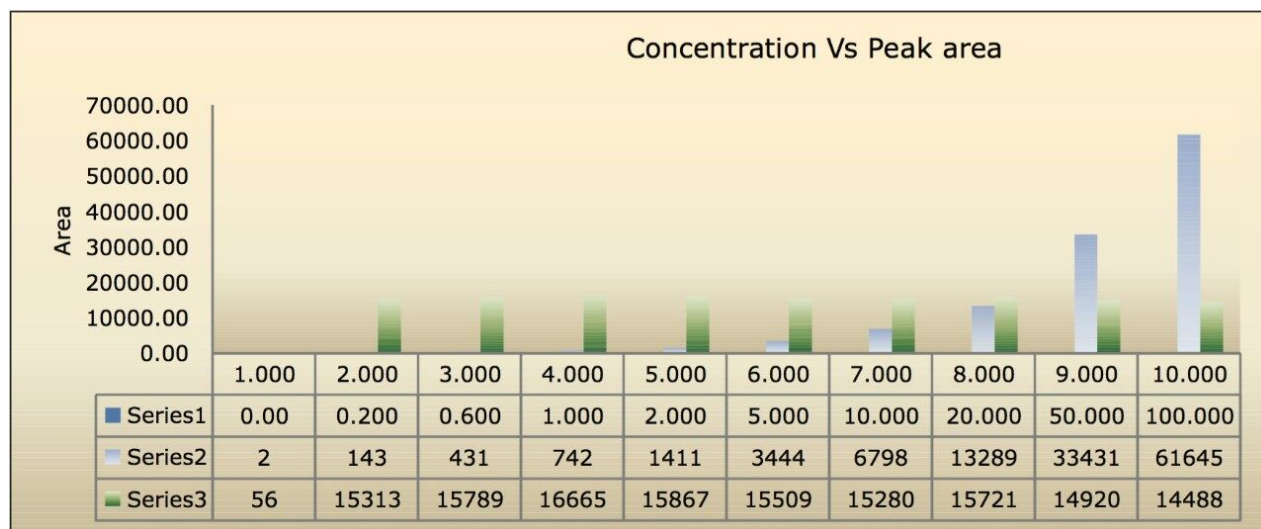

Figure 3.1. Comparison of area under curve for docetaxel (analyte) and IS for the concentration range of 200.000 pg/mL to 100.000 ng/mL.

Compound name: Docetaxel
Correlation coefficient: r = 0.999749, r^2 = 0.999498
Calibration curve: 0.0438114 * x + 0.00064206
Response type: Internal Std (Ref 2), Area * (IS Conc./IS Area)
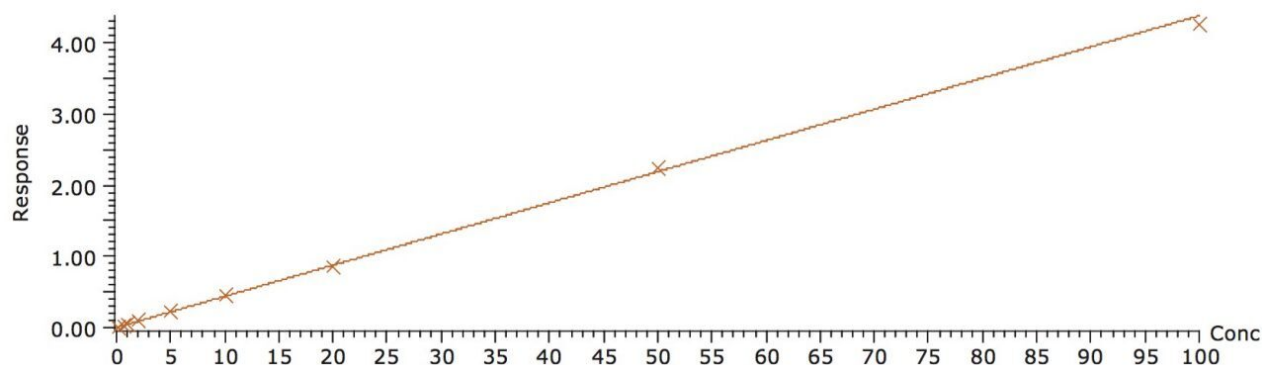Curve type: Linear, Origin: Exclude, Weighting: $1/x^2$, Axis trans: None

Figure 3.2. Calibration curve of docetaxel.

Recovery of the analyte and internal standard (IS) was performed by comparison of the extracted QC samples against six post-extracted samples, which was found to be approximately 48% at LLOQQC, LQC, MQC, and HQC levels for both analyte and the internal standard, as shown in Figures 4.1, 4.2, 4.3, and 4.4, and Table 2. The %CV for repeat batches was found to be within 10% of LLOQQC and varied between 1% to 3% for all QC levels.
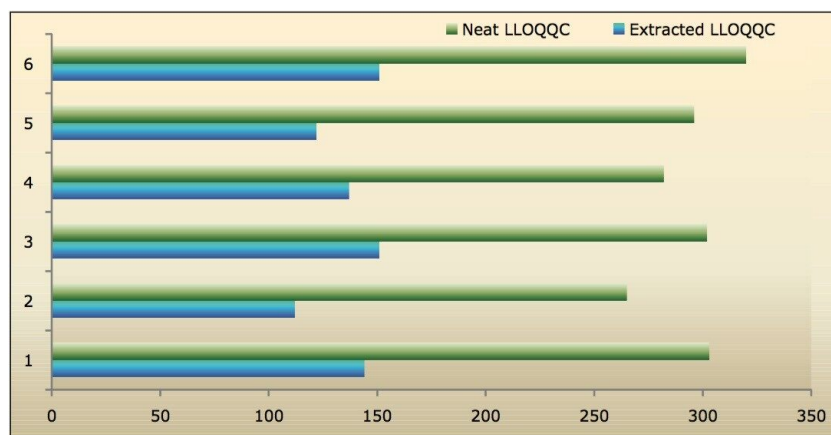

Figure 4.1. Analyte recoveries (area under the curve) from six samples of docetaxel at LLOQQC, concentrations.
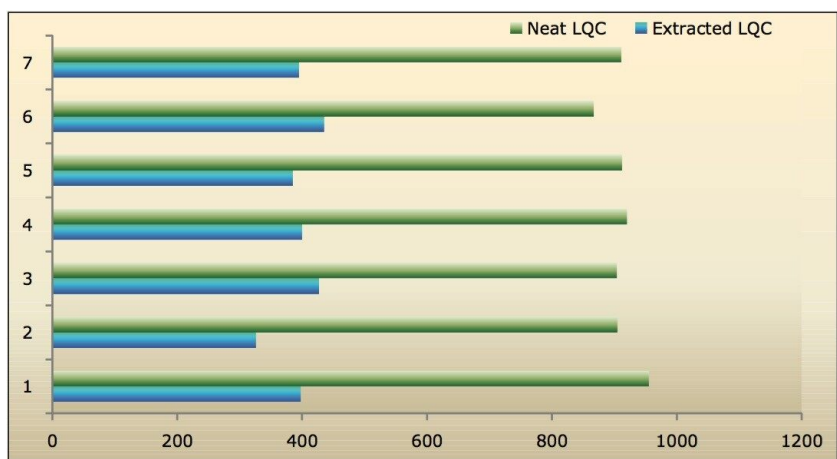
Figure 4.2. Analyte recoveries (area under the curve) from six samples of docetaxel at LQC.
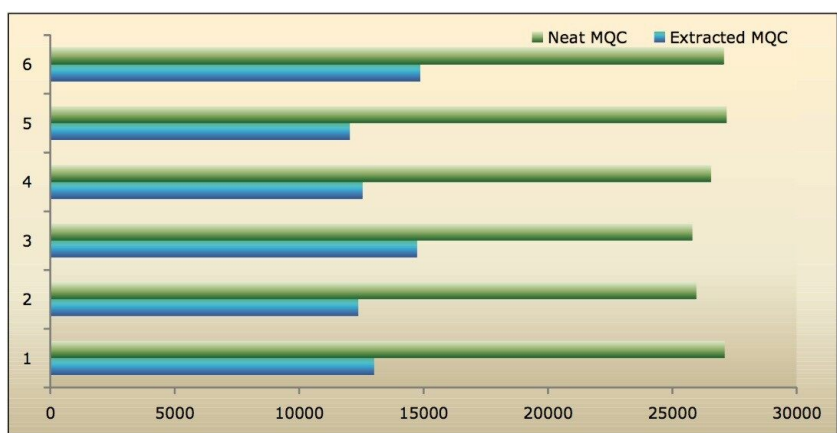

Figure 4.3. Analyte recoveries (area under the curve) from six samples of docetaxel at MQC, concentrations.
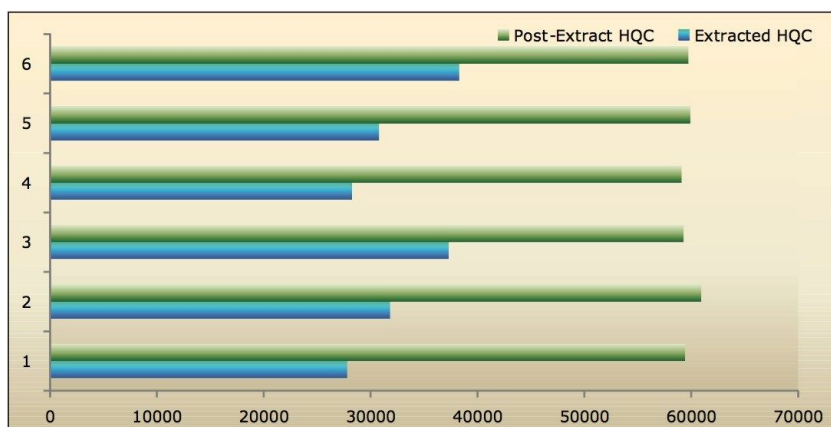
Figure 4.4. Analyte recoveries (area under the curve) from six samples of docetaxel at HQC, concentrations.

LLOQQC	LQC	MQC	HQC
46.21	43.4	49.84	54.2
Mean Analyte Recovery (%) = 48			

Table 2. Mean analyte recovery (%) of docetaxel at LQC, MQC, and HQC levels.

Data shown in Figures 4.1, 4.2, 4.3, and 4.4 shows that the analyte recovery values for the six samples did not vary significantly for any of the four concentration levels (LLOQQC, LQC, MQC, and HQC). In addition, as detailed in Table 2, the mean analyte recovery for the three concentration ranges was 48%.

For a comparison of samples within the global batches, three separate batches were prepared with six samples in each batch for LLOQQC, LQC, MQC, and HQC concentration levels. The data showed excellent agreement between the six samples for all three batches, as shown in Table 3. The mean accuracy obtained for all the samples levels was found to be > 93% for every concentration, shown in Table 3.

P-A BATCH-GLOBAL												
P-A-Batch-01	EXT_LLOQQC_1	0.2	0.1910	EXT_LQC_1	0.6	0.5740	EXT_MQC_1	20.0	20.631	EXT_HQC_1	50.0	51.8900
	EXT_LLOQQC_2	0.2	0.2010	EXT_LQC_2	0.6	0.4950	EXT_MQC_2	20.0	19.965	EXT_HQC_2	50.0	48.0810
	EXT_LLOQQC_3	0.2	0.1840	EXT_LQC_3	0.6	0.6280	EXT_MQC_3	20.0	21.092	EXT_HQC_3	50.0	51.4230
	EXT_LLOQQC_4	0.2	0.1490	EXT_LQC_4	0.6	0.5100	EXT_MQC_4	20.0	20.103	EXT_HQC_4	50.0	46.7290
	EXT_LLOQQC_5	0.2	0.2260	EXT_LQC_5	0.6	0.6910	EXT_MQC_5	20.0	21.069	EXT_HQC_5	50.0	51.4150
	EXT_LLOQQC_6	0.2	0.1960	EXT_LQC_6	0.6	0.5020	EXT_MQC_6	20.0	19.922	EXT_HQC_6	50.0	47.0610
P-A-Batch-02	EXT_LLOQQC_1	0.2	0.2430	EXT_LQC_1	0.6	0.6270	EXT_MQC_1	20.0	20.045	EXT_HQC_1	50.0	48.2270
	EXT_LLOQQC_2	0.2	0.1570	EXT_LQC_2	0.6	0.5020	EXT_MQC_2	20.0	19.880	EXT_HQC_2	50.0	48.8620
	EXT_LLOQQC_3	0.2	0.1940	EXT_LQC_3	0.6	0.5640	EXT_MQC_3	20.0	19.711	EXT_HQC_3	50.0	53.2100
	EXT_LLOQQC_4	0.2	0.2280	EXT_LQC_4	0.6	0.6220	EXT_MQC_4	20.0	19.243	EXT_HQC_4	50.0	49.0250
	EXT_LLOQQC_5	0.2	0.1720	EXT_LQC_5	0.6	0.5870	EXT_MQC_5	20.0	18.716	EXT_HQC_5	50.0	45.7640
	EXT_LLOQQC_6	0.2	0.1930	EXT_LQC_6	0.6	0.5780	EXT_MQC_6	20.0	19.839	EXT_HQC_6	50.0	53.6150
P-A-Batch-03	EXT_LLOQQC_1	0.2	0.1760	EXT_LQC_1	0.6	0.5880	EXT_MQC_1	20.0	20.133	EXT_HQC_1	50.0	48.0590
	EXT_LLOQQC_2	0.2	0.1750	EXT_LQC_2	0.6	0.4740	EXT_MQC_2	20.0	16.664	EXT_HQC_2	50.0	48.2460
	EXT_LLOQQC_3	0.2	0.1460	EXT_LQC_3	0.6	0.6230	EXT_MQC_3	20.0	18.759	EXT_HQC_3	50.0	46.7940
	EXT_LLOQQC_4	0.2	0.2080	EXT_LQC_4	0.6	0.6830	EXT_MQC_4	20.0	20.575	EXT_HQC_4	50.0	49.5330
	EXT_LLOQQC_5	0.2	0.1630	EXT_LQC_5	0.6	0.6200	EXT_MQC_5	20.0	17.674	EXT_HQC_5	50.0	45.8490
	EXT_LLOQQC_6	0.2	0.1630	EXT_LQC_6	0.6	0.5810	EXT_MQC_6	20.0	20.476	EXT_HQC_6	50.0	50.6840
	Mean		0.1870			0.5800			19.572			49.7840
	SD		0.0275			0.0455			0.500			3.0471
	%CV		14.7000			7.8500			2.550			6.1200
	Accuracy		93.4700			96.6700			97.860			99.5700

Table 3. Comparison of the three separate batches, each containing six docetaxel samples at the LLOQQC, LQC, MQC, and HQC concentrations.

Conclusion

Micro-elution offered a better solution for extraction, which employed low plasma volumes, thereby reducing matrix effect, followed by enrichment of the samples for proper extraction. The sample cleanup was found to be very effective, which can be seen with the S/N ratio obtained for LLOQ (200 pg/mL). The analyte recovery varied very little through the entire range of the calibration including the LLOQ. Excellent reproducibility was observed after summing up of two traces. Thus the above method can be used for the estimation of docetaxel in human plasma with the ACQUITY UPLC System and ACQUITY TQD.

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