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A Liquid Chromatographic-Tandem Mass Spectrometric (LC-ESI-MS/MS) Method for the Determination of Free Doxorubicin and Liposomal Doxorubicin in K3EDTA Human Plasma

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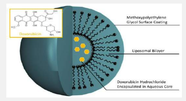
Introduction:

Doxorubicin (liposomal) is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Doxorubicin (liposomal) is used to treat AIDS-related Kaposi's sarcoma, breast cancer, ovarian cancer, and other solid tumors. Doxorubicin (liposomal) is the drug doxorubicin encapsulated in a STEALTH® liposome (Figure 1). By enclosing a drug in a STEALTH® liposome, it is able to get close to the tumor and the encapsulated drug doxorubicin becomes available to work against the tumor cells. Liposomal doxorubicin have proven to be as effective and less toxic when compared face to face with conventional doxorubicin, allowing a longer period of treatment and a higher cumulative dose of the doxorubicin (Figure 1)



Reason for accurate measurement of free doxorubicin and Liposomal doxorubicin

1. Liposomes induce greater doxorubicin accumulation in tumours compared to free drug and Liposomes slowly release doxorubicin keeping systemic drug concentration low
2. Liposomes improve doxorubicin therapeutic effect by reducing systemic peak free doxorubicin concentrations while enhancing doxorubicin AUC within the tumour

More than 95% of the doxorubicin is encapsulated in the liposomes at all times after intravenous infusion.

Challenges for Free doxorubicin and Liposomal doxorubicin method:

1. Leaching of doxorubicin from liposomal doxorubicin
2. Bursting of liposomes during thawing of samples
3. uniformity of sample and reproducibility of sample results
4. Accurate measurement of free doxorubicin in presence of Liposomal Doxorubicin
5. Accurate measurement of Liposomal doxorubicin in presence of free doxorubicin

Experimental:

Free Doxorubicin and Liposomal doxorubicin was separated using a solid phase extraction method from 200 µL of human plasma (Figure 2).

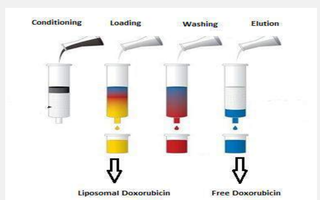


Table 1 Summary of instrumental analytical conditions

	Parameter
Column	Kinetex® 5µm Biphenyl 100 Å, 100 x 4.6 mm
Mobile Phase	A: 0.01% Formic Acid in 1mM Ammonium Acetate in water B: Acetonitrile
Mobile Phase Programme	Isocratic
Run Time	3.20 Minutes
MS Interface	ESI, positive
MS Mode	MRM Doxorubicin m/z 544.3>397.3 Doxorubicin 13C D3 m/z 548.2>401.1

RESULTS

All Validation experiments of free doxorubicin were performed using API QC and MIX QC.
All Validation experiments of Liposomal doxorubicin were performed using liposomal QC and MIX QC.

Table 2 Summary of Calibration Curve Range

Analyte	Calibration Range
Free doxorubicin	10.0 to 2000 ng/mL
Liposomal doxorubicin	150.0 to 60, 000 ng/mL

Table 3 Summary of Validation parameters for Free Doxorubicin

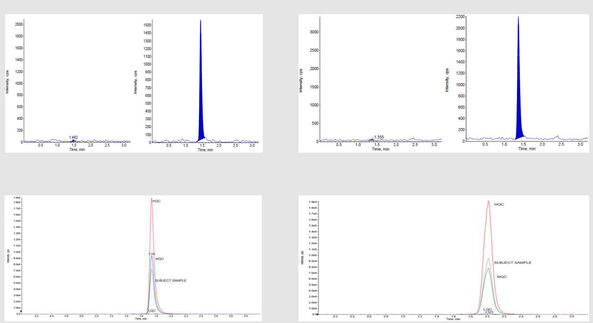
Validation Parameter	Details
Intra-batch precision (% CV)	1.11-4.40 %
Inter-batch precision (% CV)	1.52-5.82 %
Intra-batch (% bias)	-2.29-10.27 %
Inter-batch (% bias)	-1.70-3.71 %
Specificity	≤ 1.47 for doxorubicin and ≤ 0.03 for Doxorubicin 13CD3
Matrix Factor	0.995 to 1.019, %CV – 4.33
Extraction Efficiency (Recovery)	98.00%
Sensitivity	10.0 ng/mL, Accuracy (104%) and Precision (CV = 5.82%)

Table 4 Summary of Validation parameters for Liposomal Doxorubicin

Validation Parameter	Details
Intra-batch precision (% CV)	1.46-5.61 %
Inter-batch precision (% CV)	2.94-9.46 %
Intra-batch (% bias)	-2.67-5.90 %
Inter-batch (% bias)	-9.06-3.07 %
Specificity	≤ 1.95 for doxorubicin and ≤ 0.06 for Doxorubicin 13CD3
Matrix Factor	1.005 to 1.015, %CV – 1.28
Extraction Efficiency (Recovery)	66.37%
Sensitivity	150.0ng/mL, Accuracy (103%) and Precision (CV = 9.46%)

Figure 3: Representative chromatograms of blank plasma sample and lowest standard spiked into blank plasma

Free Doxorubicin | **Liposomal Doxorubicin**



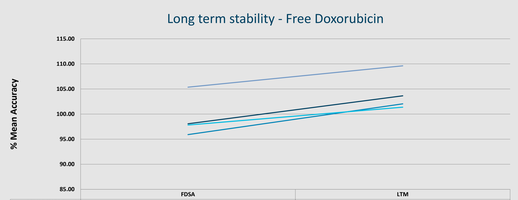
Stability

Stability was evaluated using spiked samples at two QC levels and using both type of QC (API and Mix QC for free doxorubicin, Liposomal and MIX QC for Liposomal doxorubicin).

Table 5: Stability parameters and the detailed conditions examined

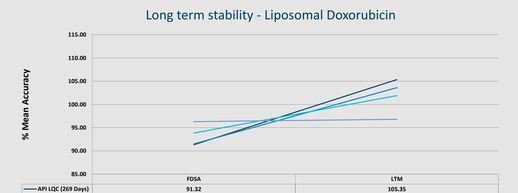
Parameter	Conditions
Bench top stability in plasma	04 h ≤8°C temperature under normal light for free doxorubicin and Liposomal doxorubicin
Freeze thaw stability in plasma	Three freeze thaw cycles at -78 °C for free doxorubicin and Liposomal doxorubicin
Auto sampler stability	78 h at 5 °C and 24 h at ambient temperature for free doxorubicin and Liposomal doxorubicin
Stability at -20±5 °C	36 Hours at -20±5 °C for free doxorubicin and Liposomal doxorubicin
Long term stability at -55±8 °C	25 Days at -55±8 °C for free doxorubicin and Liposomal doxorubicin
Long term stability at -78±8 °C	179 days for free doxorubicin and 268 days for Liposomal doxorubicin at -78±8 °C

Long term stability - Free Doxorubicin



	FDSA	LTM
API HQC(181 Days)	95.90	102.04
API LQC(181 Days)	98.07	103.64
MIX HQC(179 Days)	97.82	101.37
MIX LQC(179 Days)	105.36	109.62

Long term stability - Liposomal Doxorubicin



	FDSA	LTM
API LQC (269 Days)	91.32	105.35
API HQC(269 Days)	91.51	103.61
MIX HQC(268 Days)	93.83	101.90
MIX LQC(268 Days)	96.31	96.80

Batch Size Experiment (BSE) – Free Doxorubicin

Details	Total 137 samples including Calibration Curve	
	API (LQC to HQC)	MIX (LQC to HQC)
Precision (%CV)	0.73 to 1.74	1.99 to 7.10
% Mean Bias	-1.09 to 4.91	-0.64 to 3.91

Batch Size Experiment (BSE) – Liposomal Doxorubicin

Details	Total 135 samples including Calibration Curve	
	API (LQC to HQC)	MIX (LQC to HQC)
Precision (%CV)	0.08 to 1.74	1.31 to 3.40
% Mean Bias	-1.95 to 2.92	-8.23 to -4.45

Conclusion

Two separate LC-MS/MS assay has been developed to measure free doxorubicin and liposomal doxorubicin in plasma. SPE cartridges effectively separated free and liposomal doxorubicin from plasma. Accuracy, precision and stability results indicate that the both assay are reproducible, robust and no any leaching of doxorubicin from liposomes during sample processing and storage. The method can be applied to clinical analysis of pharmacokinetic studies of generic liposomal formulations of doxorubicin.

References

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