Determination of Free doxorubicin and Liposomal doxorubicin in human plasma by LC-MS/MS method

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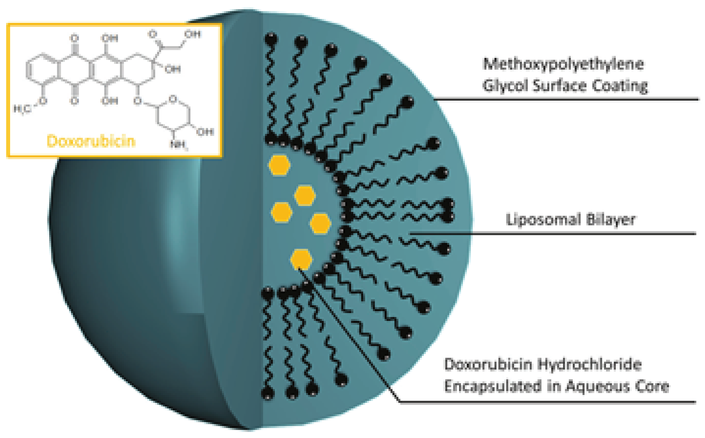
Introduction:

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)**. Liposomes are closed lipid spheres made of the basic components of natural human cell walls. The STEALTH® liposome have on their surface a substance to protect the liposome from detection by the body's immune system and to increase the time this medication is circulating in the blood. 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

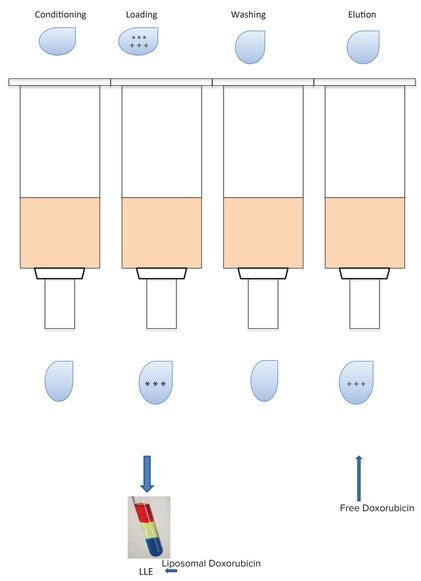
1. Liposomes induce greater doxorubicin accumulation in tumours compared to free drug (Gabizon 1994, Laginha 2005)
2. Liposomes slowly release doxorubicin keeping systemic drug concentration low (Gabizon 1994, Laginha 2005)
3. Liposomes improve doxorubicin therapeutic effect by reducing systemic peak free doxorubicin concentrations while enhancing doxorubicin AUC within the tumour (Gabizon 1994, Amantea 1997, Laginha 2005)

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

Experimental:

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

Figure 2



The extracted sample was analysed on system consisting of the Shimadzu LC-20AD HPLC and the API 4000 mass spectrometer. A summary of the instrumental conditions are presented in Table.

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
MS Interface	ESI, positive
MS Mode	MRM Doxorubicin m/z 544.3>397.3 Doxorubicin 13C D3 m/z 548.2>401.1

The analytical developed was validated for parameters including linearity, sensitivity, selectivity, matrix effect, extraction efficiency and stability under different storage conditions.
Using the analytical conditions developed, doxorubicin and its ISTD could be rapidly analysed as they eluted at approximately 1.60 min.

Results and Discussion

Linearity

The calibration curve was constructed using 8 calibration standards ranging from 10.0 to 2000 ng/mL for free doxorubicin and 150.0 to 60,000 ng/mL for Liposomal doxorubicin. The curve fitted well to linear regression (R2 > 0.998).
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.

Accuracy and Precision

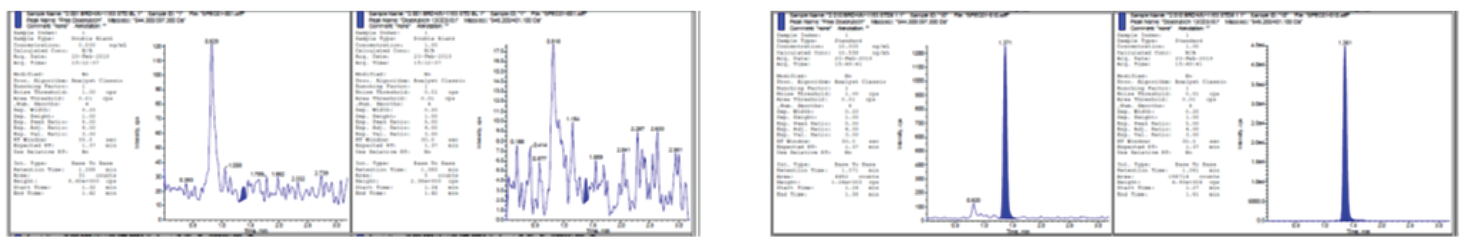
Free Doxorubicin: The intra-batch and inter-batch precision (% CV) varied from 1.11-4.40 % and 1.52-5.82 % respectively and intra-batch and inter-batch bias (% bias) varied -2.29-10.27 % and -1.70-3.71 % respectively
Liposomal Doxorubicin: The intra-batch and inter-batch precision (% CV) varied from 1.46-5.61 % and 2.94-9.46 % respectively and intra-batch and inter-batch bias (% bias) varied -2.67-5.90 % and -9.06-3.07 % respectively

Selectivity

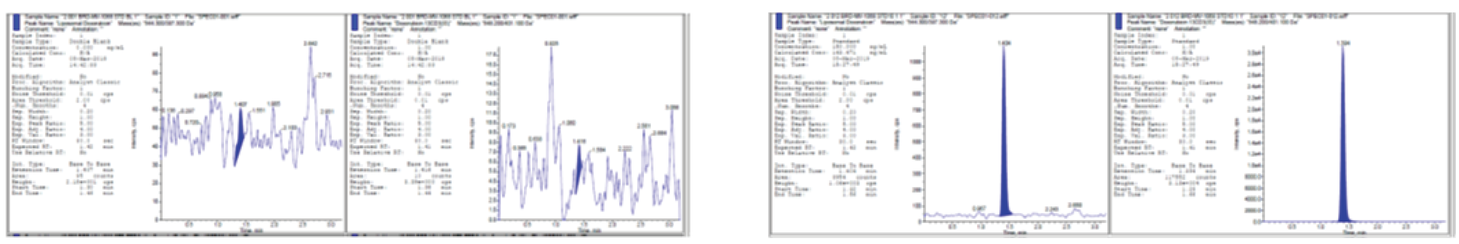
The method was found to be highly selective for the analytes as blank chromatograms from normal, lipemic and hemolyzed plasma showed no significant interference at the retention times and MRM window of Doxorubicin and its ISTD for both method (Figure 3).

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

Free doxorubicin



Liposomal doxorubicin



Matrix effect

Matrix effect did not exert a significant effect in this analysis as the matrix factor for doxorubicin and its internal standard were 0.995 and 1.019 respectively.

Extraction efficiency

The extraction efficiency of the sample preparation method was determined to be 98.6% for free doxorubicin and 65.0% for Liposomal doxorubicin.

Repeatability

The method showed extremely good repeatability as consecutive injections of QC samples showed CV of less than 5%.

Sensitivity

At the LLOQ concentration of 10 ng/mL for free doxorubicin, the analyte was detected with high accuracy (104%) and precision (CV = 5.82%).
At the LLOQ concentration of 150 ng/mL for Liposomal doxorubicin, the analyte was detected with high accuracy (103%) and precision (CV = 9.46%).

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). The samples did not show significant degradation under the conditions examined (Table 3).

Table 2: Stability parameters and the detailed conditions examined

Parameter	Condition
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
Autosampler stability	78 h at 5 °C 24 h at ambient temperature for free doxorubicin and Liposomal doxorubicin
Long term stability	179 days for free doxorubicin and 268 days for Liposomal doxorubicin at -78±8 °C

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

Liu, Y., Yang, Y., Liu, X., & Jiang, T. (2008). Quantification of pegylated liposomal doxorubicin and doxorubicinol in rat plasma by liquid chromatography/electrospray tandem mass spectroscopy: Application to preclinical pharmacokinetic studies. Talanta, 74(4), 887-895.