Doxoves™ - Liposome Doxorubicin Compared to Doxil®

Many customers have asked us how our liposome doxorubicin HCl, Doxoves™, compares to the commercial product Doxil®. Doxoves™ is prepared by senior formulation scientists who are very experience in the liposome technology used for Doxil®. Doxoves™ contains the same lipid composition and drug/lipid ratio. Liposome down-sizing is achieved by extrusion using a Lipex extruder, and the transmembrane ammonium gradient is established by diafiltration, the proper technique for large scale manufacturing of liposome products. Doxoves™ exhibits comparable physical characteristics regarding particle size (approx. 85nm), narrow size distribution, and drug encapsulation efficiency (>98%), and uses the same bulk buffer solution (10wt% sucrose, 10mM histidine, pH 6.5). Doxoves™ is sterile filtered and filled in autoclaved glass vials for long-term stability. However, as an added benefit Doxoves™ is provided at 4mg/mL drug concentration (compared to 2mg/mL Doxil®) in order to allow researchers to conduct experiments at much higher drug concentrations/doses.

Below you will find some results which we have obtained from research with Doxoves™. Cryo-TEM conducted at NanoImaging Services showed consistent particle size, predominantly unilamellar structure and the rod-like structures of the doxorubicin/sulfate co-crystals inside the liposomes. Results from a recent PK study in rats showed a similar plasma drug PK profile to that of Doxil® with a plasma drug half-life of 30 - 40 hrs. The two batches of Doxoves™ showed identical PK profiles and parameters. You will also find a typical certificate of analysis of Doxoves™ on page 4.

However, please keep in mind that although we have confidence in the quality of Doxoves™, it nevertheless is a research grade product. It is not manufactured under cGMP conditions. We have not conducted any investigations regarding its biodistributions in tissues and/or in tumors, toxicity and/or antitumor efficacy in house or sponsored by FormuMax.

Comparison of characteristics of Doxoves and Doxil

Parameters	Doxil®/Caelyx®	Doxoves™
Lipid composition	HSPC/CHOL/mPEG2000-DSPE (56.3:38.4:5.3 mol%)	HSPC/CHOL/mPEG2000-DSPE (56.3:38.4:5.3 mol%)
Lipid concentration	20mM	40mM
Doxorubicin HCl	2mg/mL	4mg/mL
Drug encapsulation	High (>95%)	High (>98%)
Loading battery	Ammonium sulfate 250mM	Ammonium sulfate 250mM
External buffer	10% sucrose, 10mM histidine, pH 6.5	10% sucrose, 10mM histidine, pH 6.5
Particle size (diameter)	80-85 nm	80-85 nm
Manufacture process	See appreciate references	Extrusion, diafiltration and active loading of drug. Final product sterile filtered
Product grade	Commercial (FDA approved)	Research use only

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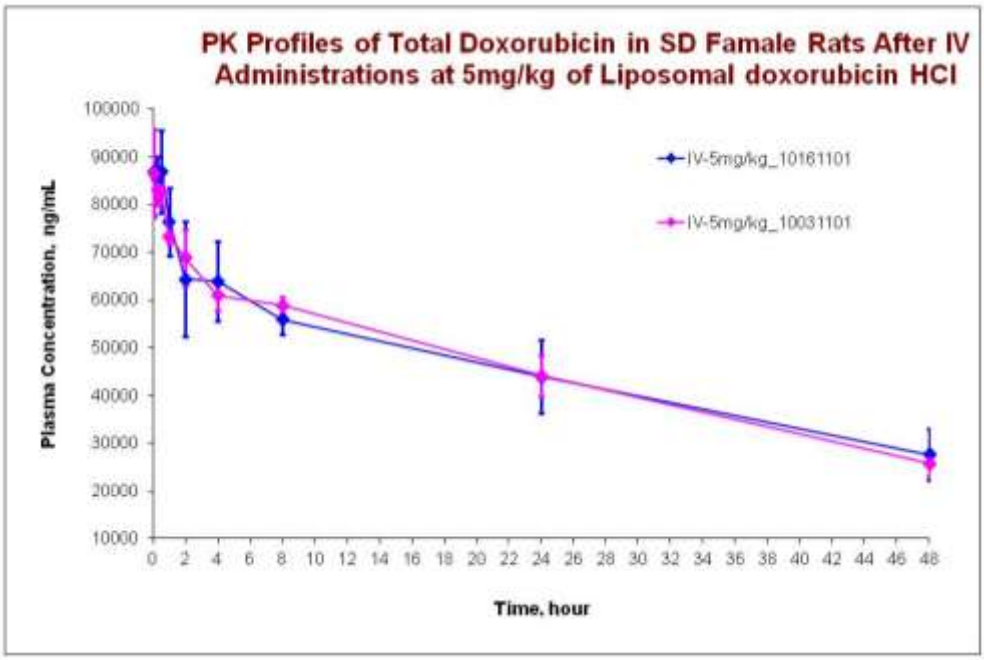
Pharmacokinetic studies of Doxoves™ in Rats



SD female rats were dosed at 5mg/kg intravenously (jugular vein cannulation). Total doxorubicin concentration in plasma was assayed by HPLC-MS/MS.

Group	Animal ID	T1/2	Tmax	Cmax	AUC0-t	AUC0-inf	Vz	CL	MRT0-in	Vss
		hour	hour	μg/mL	hour*μg/mL	hour*μg/mL	mL/kg	mL/hour/kg	hour	mL/kg
Batch 10161101	1	43.2	0.25	90.2	2345	4234	73.6	1.2	60.3	71.2
	2	31.1	0.08	85.6	1855	2816	79.7	1.8	43.9	78.0
	3	43.5	0.08	96.5	2342	4292	73.0	1.2	61.0	71.1
	mean	**39.3**	**0.14**	**91**	**2181**	**3781**	**75.5**	**1.37**	**55.1**	**73.4**
	SD	**7.1**	**0.10**	**5**	**282**	**835.8**	**3.7**	**0.348**	**9.68**	**3.93**
Batch 10031101	1	33.3	0.08	82.663	2113	3333	72	1.5	52	71
	2	38.1	0.25	84.421	2302	3856	71	1.3	59	69
	3	29.0	0.08	97.063	2140	3120	67	1.6	45	65
	mean	**33.5**	**0.14**	**88**	**2185**	**3436**	**70.2**	**1.47**	**51.8**	**68.5**
	SD	**4.6**	**0.10**	**8**	**102**	**378**	**2.7**	**0.16**	**6.9**	**3**

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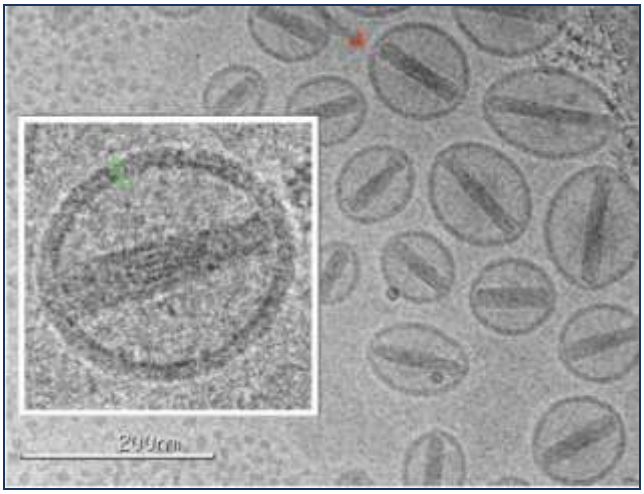
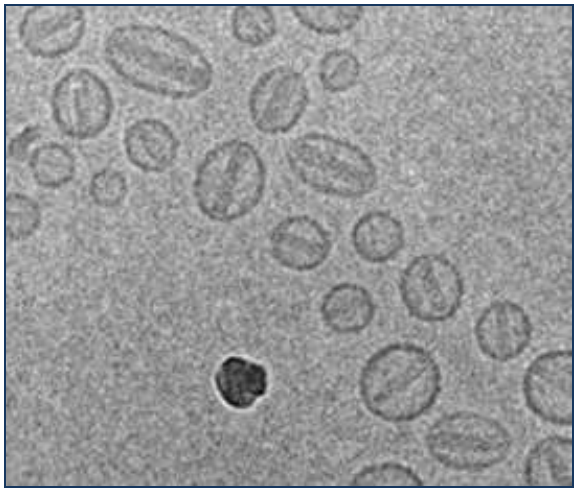
Structural Comparison of Doxoves™ and Doxil® studied by Cryo-TEM



Cryo-TEM of Doxoves™
(Cryo-TEM Acquired by Nanoimaging Services)



Cryo-TEM of Doxil® (link to origin)

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CERTIFICATE OF ANALYSIS

Product Is Cytotoxic And Handle With Care! Product Is For Research Only, Not For Human Use!

DESCRIPTION:	**Doxoves ™- Liposomal Doxorubicin HCl**
CATALOGUE #:	**F30204B-D**
BATCH #:	10171101
VOLUME:	300 mL
DATE OF PRODUCTION:	10/20/2011
LIPID COMPOSITION:	HSPC/CHOL/mPEG2000-DSPE (56.3:38.4:5.3 mol%)
ACTIVE:	Doxorubicin HCL

ANALYTICAL DATA:

Lipid concentration:	42.0 ± 0.3 mM (31.2 ± 0.2 mg/mL) (Stewart assay)
Drug concentration:	4.15 ± 0.11 mg/mL (UV)
Free Drug concentration:	0.02 mg/mL (filtration/UV)
Drug encapsulation efficiency:	>99.0% (calculated from free drug and total drug concentrations)
Hydration solution (battery):	250 mM ammonium sulfate
External buffer solution:	10 wt% sucrose, 10mM histidine pH 6.5
Particle size (ZetaPALS):	Mean diameter: 82.7 ±1.2 nm; Half-width: 22.7±2.5nm, Polydispersity: 0.08±0.03
Zeta Potential (ZetaPALS):	-23.0 ± 1.2 mV (measured in 1mM NaCl)

Form/Color:	Translucent, red and free flow liposomal dispersion, no visible particles/aggregates with naked eye or under microscope
Stability:	Product is sterile filtered (0.2μm) and filled in autoclaved vials. Free from bacteria growth for 3 month for unopened vials stored at 2 – 8 ºC.

STORAGE, HANDLING AND CLEANING:

- Store refrigerated (2-8 deg C). **Avoid freezing**. Warm to room temperature before use. Visually exam the uniformity of the product. Mix before opening the vial.
- **Method of cleaning:** alcohol/bleach/detergent mixture.

HSPC: fully hydrogenated phosphatidylcholine
CHOL: cholesterol
mPEG2000-DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]

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